MAY, 1948 RESEARCH BULLETIN 417

UNIVERSITY OF MISSOURI COLLEGE OF AGRICULTURE

AGRICULTURAL EXPERIMENT STATION

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# THE THYROID SECRETION RATE IN THE MOUSE AND ITS RELATION TO VARIOUS PHYSIOLOGICAL PROCESSES

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Publication authorized May 12, 1948

COLUMBIA, MISSOURI

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# THE THYROID SECRETION RATE IN THE MOUSE AND ITS RELATION TO VARIOUS PHYSIOLOGICAL PROCESSES

Victor Hurst and C. W. Turner

## INTRODUCTION

In the development of a technique for measuring the thyroid secretion rate of an animal, Dempsey and Astwood (1943) and Mixner et al., (1944) have given us an important tool for studying quantitatively the relation of thyroid secretion to various physiological functions. Investigators have long appreciated the importance of the thyroid gland as a regulator of many body processes, and in their attempts to understand the relationship of the thyroid gland to these processes they have created hypothyroid conditions by thyroidectomy or the administration of goitrogens, and they have produced hyperthyroidism by the administration of exogenous thyroid hormone.

Many of these workers who produced hypothyroid and hyperthyroid conditions have failed to take into account the degree to which they were altering thyroid hormone supply in terms of the animal's own thyroid secretion rate. As a result there has been much controversy in regard to the general effects of hyperthyroidism. Many workers have caused hyperthyroidism by giving massive dosages of thyroid hormone; dosages that we now know to be outside the range of normal physiological secretion. Such investigators believe hyperthyroidism to be synonymous with a "toxic" condition, and they indicate that thyroid administration is poisoning the animal.

Other workers, producing mild forms of hyperthyroidism, have shown that small dosages of thyroid hormone will accelerate growth rates and increase milk and egg production. These workers consider mild hyperthyroidism beneficial to many species of animals.

Unfortunately, it is not generally realized that the workers who think in terms of hyperthyroidism as being "toxic" and those who claim that it can be beneficial, may both be talking simply of different degrees of the same function. The value of determining the thyroid secretion rate in animals is that under given environmental conditions a reference base could be established, and then investigators could report their work in terms that would be understood in other laboratories; namely, how much they have altered the thyroid hormone supply in relation to the animal's own thyroid secretion rate.

This paper is concerned with the determination of the thyroid secretion rate and its relation to physiological function in the laboratory mouse. The mouse has been used extensively for the study of thyroid physiology, and the results presented here should clarify some of the heretofore controversial results arising from choice of thyroid dosage levels, and at the same time provide a reference base for future work.

Before the experimental work can be presented, however, it will first be necessary to define thyroid secretion rate and to describe its measurement.

## THE THYROID SECRETION RATE

The thyroid secretion rate is the amount of thyroid hormone that is being secreted into the general circulation by the thyroid gland in a given period of time under defined environmental conditions. It is determined by the amount of exogenous thyroid hormone required, when administered simultaneously with a goitrogen, to maintain a thyroid weight which is the same, on a body weight basis, as that of control animals over a given period of time under similar environmental conditions.

The mechanism of this assay method is easily explained if we consider the two main factors involved; namely, the physiology of the thyroid gland and the action of the goitrogens, and then show how these factors can be interrelated to give us a measurement of thyroid secretion rate.

### The Anatomy of the Thyroid Gland

The thyroid is an endocrine gland, embryologically derived from the pharynx and therefore of entodermal origin. Grossly it consists of two lobes connected ventrally by a thin isthmus. The lobes lie on the lateral surfaces of the trachea just caudad to the larynx. The thyroid has a rich vascular and lymphatic supply, and is innervated by the vagi (parasympathetic) nerves, and by the cervical sympathetic nerves.

Within the thyroid lobes are masses of follicles, separated from one another by interfollicular tissue. Each follicle is a separate unit consisting of a lumen surrounded by basal epithelial cells.

### The Physiology of the Thyroid Gland

Factors Responsible for the Secretion and Release of the Thyroid Hormone. Numerous external factors such as temperature, light, and humidity, and internal factors such as exercise, food consumption, growth, pregnancy, lactation, and senescence are indirectly concerned with the secretion and release of the thyroid hormone. Each of these factors, in turn, influences more direct factors, the nervous and endocrine systems, whose relation to the secretion and release of the thyroid hormone will now be described.

Nervous control. The general concept at this time is that there is no direct nervous control of the thyroid gland but there is evidence that by controlling the supply of blood to and from the thyroid gland, the nervous system may play some indirect role in thyroid physiology.

Uotila (1939) showed that the cervical sympathetics do not play a part in the function of the rat thyroid. In the guinea pig, Lowe et al., (1945) reported that bilateral cervical sympathectomy did not modify the metabolic response of the thyroid to injected thyrotrophic hormone. Removal of the cervical sympathetics did, however, cause an increased metabolic response to cold. The authors reasoned that inasmuch as the cervical sympathetics act as vasoconstrictors, the vasoconstricting power had been lost and with it an ability to restrict the output of thyrotrophin or thyroxine from their glands of origin through the blood.

Aleskin and Sarenko (1947) measured the oxygen consumption of thyroid tissue. Acetylcholine added to the medium retarded oxygen consumption, and adrenalin increased it. Soffer et al., (1947) observed hy-

perplastic changes in the dog thyroid following the injection of adrenalin-in-oil. If the dogs were thyroidectomized, the injection of adrenalin-in-oil resulted in a marked increase of blood thyrotrophin.

Endocrine control. Direct endocrine control of the thyroid gland results from a reciprocal pituitary-thyroid relationship. The pituitary, an endocrine gland located at the base of the brain, is composed of the anterior, intermediate, and posterior lobes. Our concern is with the anterior lobe, or hypophysis, whose basophilic cells secrete the thyrotrophic hormone.

Following its release from the pituitary gland, the thyrotrophic hormone stimulates its target organ, the thyroid gland. It stimulates the synthesis of the thyroid hormone and also is necessary for the release of the formed thyroid hormone from the thyroid gland.

The cycle of reciprocal regulation is completed by the circulating thyroid hormone acting back on the pituitary gland with large amounts depressing and small amounts stimulating the secretion and release of the thyrotrophic hormone.

## Action of the Goitrogens

Goitrogens, by definition, are substances capable of producing an enlarged thyroid gland. They occur in plants such as in the seeds and leaves of the Brassica family, the soy bean, ground-nut, wheat, and oats, and they are also found in the chemical forms of analine derivatives, thiourea and its derivatives, and thiocyanates.

Goitrogenic Effects Directly Concerned with Thyroid Secretion Rate. The part played by the goitrogens in the measurement of thyroid secretion rate is based on their ability to prevent the synthesis of thyroxine by the thyroid and the subsequent effects of a lowered circulating thyroid hormone on pituitary-thyroid relationship.

Prevention of thyroxine synthesis. Franklin and Chaikoff (1944) found that sulfanilamide, sulfathiazole, sulfapyridine, sulfaguanidine, and sulfadizine prevented the in vitro formation of diiodotyrosine and thyroxine in the presence of radioactive iodine ($I^{131}$). Thiouracil, thiourea, thiocyanate, para-aminobenzoic acid, and para-aminophenylacetic acid also prevented thyroid slices from synthesizing diiodotyrosine or thyroxine in the presence of radio-active iodine. (Franklin et al., 1944).

The next step, to solve the means by which the goitrogens prevent the formation of thyroxine, has yet to be accomplished. Most investigators have worked on this problem by the study of enzyme systems. Lerner and Chaikoff (1945) concluded that the goitrogens did not affect cytochrome-oxidase system in the thyroid, and Glock (1946), has not been able to demonstrate the presence of a peroxidase system in the thyroid. If thiouracil is administered to rats, (Tipton and Nixon, 1946), the activities of the succinoxidase and cytochrome-oxidase systems in the liver are lowered. This action of thiouracil is an indirect one, however, because in culture media this work could not be repeated. It was concluded that the action of the oxidase systems declined because of lowered thyroid activity. This same mechanism may have played a part in the observations of Tissieres (1946) who reported that thyroidectomy or methylthiouracil administration in rats reduced the cytochrome content of the muscles of the hind leg.

Pituitary-thyroid relationship. From a previous discussion it is apparent that if any part of the pituitary-thyroid relationship is disturbed, there is a compensation by the other factors concerned in an attempt to maintain a normal balance. Inasmuch as preventing thyroxine formation would subsequently lower its content in the blood, one would expect that goitrogen administration would result in marked changes in the pituitary and in the amount of thyrotrophic hormone secreted.

(a) Pituitary changes: Sharpless and Hopson (1940) fed soy bean meal to rats and observed an increase in pituitary basophils, the cells which secrete the thyrotrophic hormone, and a decrease in the acidophils. These changes could be prevented by the simultaneous administration of iodine or desiccated thyroid. Mackenzie and Mackenzie (1943) reported that the pituitaries of rats fed sulfonamides or thiourea contained increased numbers of basophils. Similarly, Gordon et al., (1945b) decreased pituitary acidophils in rats fed thiouracil or para-aminobenzoic acid. Leblond and Hoff (1944) lowered the numbers of pituitary acidophils in rats both by thyroidectomy and by thiourea feeding.

(b) Thyrotrophic hormone secretion: By observing the effects on guinea pig thyroids, Griesbach and Purves (1943) showed that the blood serum of donor rats contained increased amounts of thyrotrophin following thyroidectomy or rape seed feeding. In studying the effects of pituitaries and blood serum on tadpole metamorphosis, Gordon et al., (1945a) reported that thyroidectomy in rats increased and thiourea or sulfaguanidine decreased both pituitary and blood thyrotrophin. In this work, dosage may have been a factor, or possibly the observations of Hawson et al., (1946) in which they observed that rabbit thyroids in culture media inactivated the thyrotrophic hormone, may have played a part.

Thyroid function. The purpose of this section is to show that goitrogens, in addition to affecting the pituitary-thyroid relationship by producing pituitary changes and by augmenting the circulating thyrotrophic hormone, also affect the thyroid gland in a manner similar to large doses of injected thyrotrophic hormone.

In order to simplify the presentation of pertinent literature to be found on this subject, work reported to date is summarized in Table 1. Under the column headed "Thyroid Response," a positive response will indicate one typical of thyrotrophin stimulation; namely, increased size, hyperemia, loss of colloid, and a heightened basal epithelium. Although not all of the authors have reported on all of these responses, those who have show them all to be correlated. Any deviations from these correlated changes will be noted.

From Table 1 it will be noted that with few exceptions, goitrogens stimulated the secretion of the thyrotrophic hormone. The guinea pig responds little if at all to goitrogens, and the sulfonamides do not affect the thyroid of the chicken. If the animals are hypophysectomized, (Mackenzie and Mackenzie, 1943), the administration of goitrogens does not stimulate the thyroid gland because no thyrotrophic hormone is present.

For more complete chemical studies of the goitrogens and their effects on the thyroid gland, the reader is referred to the works of Astwood (1943), Bywater et al., (1945), McGinty and Bywater (1945a, 1945b), and Jensen and Kjerulf-Jensen (1945).

TABLE 1 -- RESPONSE OF THYROID GLANDS TO ADMINISTERED GOITROGENS

| Investigators | Goitrogen | Response | Remarks |
|---|---|---|---|
| | The Rat | | |
| McCarrison (1933) | cabbage, ground nut | positive | |
| McCarrison (1933) | soy bean, methyl cyanide | negative | |
| Hellwig (1934) | rolled oats | positive | |
| Hercus and Purves (1936) | rape seed, mustard seed, cabbage seed | positive | |
| Sharpless (1938) | soy bean | positive | |
| Sharpless and Hopson (1940) | soy bean | positive | |
| Kennedy and Purves (1941) | chou moullier, soft turnip seed, rape seed, swede seed | positive | |
| Wilgus et al. (1941) | soy bean | positive | |
| Emge and Laquer (1941) | wheat germ | positive | |
| Mackenzie et al. (1941) | sulfaguanidine | positive | |
| Mackenzie and Mackenzie (1942) | sulfadiazine, sulfapyridine, sulfanilamide, thiourea | positive | |
| Richter and Clisby (1942) | phynylthiocarbamide | positive | |
| Kennedy (1942) | thiourea | positive | |
| Whitehead (1943) | rape seed | positive | |
| Mackenzie and Mackenzie (1943) | thiourea, sulfaguanidine, sulfadiazine | positive | no results if animals hypophysectomized |
| Astwood et al. (1943) | sulfaguanidine, thiourea | positive | |
| Mackenzie and Mackenzie (1944) | sulfaguanidine | positive | drop of basal metabolic rate coincided with loss of colloid |
| Higgins and Larson (1944) | promizole | positive | |
| Astwood and Bissell (1944) | thiouracil | positive | enlarged thyroids reduced following hypophysectomy |
| Leblond and Hoff (1944) | thiourea, thiouracil, sulfathiazole, sulfadiazine | positive | lowered heart rate |
| Astwood (1945) | thiouracil | positive | |
| Higgins (1945) | promizole | positive | |
| Gordon et al. (1945b) | para-aminobenzoic acid, thiouracil | positive | |
| Astwood et al. (1945) | thiobarbital | positive | |
| Williamson et al. (1945) | thiourea | positive | ointment on wounds |
| Dempsey and Astwood (1943) | thiouracil | positive | |
| Monroe and Turner (1946) | thiouracil | positive | |
| Griesbach et al. (1945) | rape seed | positive | thyroid adenomats after feeding fifteen months |
| Higgins and Joneson (1946a, 1946b) | promizole | positive | |
| Kjerulf-Jensen (1946) | thiouracil | positive | |
| Jones (1946) | thiourea | positive | hypertrophy of golgi apparatus in epithelial cells |
| Meites and Turner (1947) | thiouracil | positive | |
| Mackenzie (1947) | sulfaguanidine, thiouracil, thiourea | positive | |

TABLE 1 (continued)

| Investigators | Goitrogen | Response | Remarks |
|---|---|---|---|
| Dvoskin (1947) | thiouracil, sulfadiazine | positive | |
| Lawson and Rimington (1947) | ergothioneine | positive | substance normally present in human blood |
| | **The Mouse** | | |
| Mackenzie and Mackenzie (1942) | sulfathiazole, sulfaguanidine, sulfapyridine | positive | |
| Mackenzie and Mackenzie (1943) | sulfaguanidine, thiourea | positive | Swiss and Carworth strains |
| Dalton et al. (1945) | thiourea | positive | C3H strain |
| Waldo (personal communication) | thiouracil | negative | C57 and dba strains |
| Freiesleben et al. (1945) | thiouracil | positive | |
| Dalton et al. (1946) | thiouracil | positive | C3H strain |
| Hurst and Turner (1947) | thiouracil | positive | Schwing strain |
| | **The Rabbit** | | |
| Chesney et al. (1928) | cabbage | positive | |
| Webster et al. (1928) | cabbage | positive | basal metabolic rate decreased |
| Webster and Chesney (1928) | cabbage | positive | |
| Marine et al. (1929) | cauliflower, cabbage, brussels sprouts | positive | |
| Marine et al. (1932) | cyanides | positive | |
| Hercus and Purves (1936) | cabbage | positive | |
| Freiesleben et al. (1945) | methylthiouracil | positive | hyperplasia but no change in thyroid weight |
| | **The Guinea Pig** | | |
| Mackenzie and Mackenzie (1943) | sulfapyridine, sulfaguanidine | negative | |
| Astwood (1945) | thiouracil | questionable | slight response in adult thyroids. smaller than controls in young animals |
| Freiesleben et al. (1945) | methylthiouracil | negative | |
| McQuillen and Trikojus (1946) | thiourea | positive | |
| | **The Dog** | | |
| Mackenzie and Mackenzie (1943) | sulfaguanidine | positive | |
| Steiner and Kendall (1946) | thiouracil | positive | |
| Mayer (1947) | propylthiouracil | positive | |
| | **The Monkey** | | |
| Engle and Aranow (1946) | thiouracil | positive | effect intensified if thyrotrophic injected simultaneously |

TABLE 1 (continued)

| Investigators | Goitrogens | Response | Remarks |
|---|---|---|---|
| | **The Cat** | | |
| McCloskey et al. (1947) | thiouracil | positive | glands appeared atrophic |
| | **The Hamster** | | |
| Harris et al. (1946) | sulfadiazine, thiouracil | positive | |
| | **Ruminants** | | |
| Schultze and Turner (1945) | thiouracil, thiourea | positive | goat, dairy calf |
| Beeson et al. (1946) | thiouracil | positive | yearling steer |
| Andrews et al. (1946) | thiouracil, thiourea | positive | lambs |
| Andrews et al. (1947) | thiouracil, thiourea | positive | lambs |
| Beeson et al. (1947) | thiouracil | positive | yearling steer |
| | **Swine** | | |
| McMillen et al. (1947) | thiouracil | positive | |
| Wallach et al. (1947) | thiouracil | positive | |
| | **The Chicken** | | |
| Patton et al. (1939) | soy bean | positive | |
| Wilgus et al. (1941) | soy bean | positive | |
| Wilgus et al. (1941) | oat groats | negative | |
| Mackenzie and Mackenzie (1943) | sulfaguanidine | negative | |
| Mixner et al. (1944) | thiourea, thiouracil | positive | |
| Astwood et al. (1944) | para-aminobenzoic acid, sulfadiazine | negative | |
| Astwood et al. (1944) | thiouracil | positive | |
| Schultze and Turner (1945) | thiourea, thiouracil | positive | |
| Andrews and Schnetzler (1945) | thiouracil | positive | |
| Turner (1946a) | rape seed, thiouracil | positive | |
| Turner (1946b) | thiouracil, thiobarbital | positive | |
| Vanderlaan and Bissell (1946) | sulfaguanidine, sulfadiazine | slight effect | |
| Andrews and Schnetzler (1946) | thiouracil | positive | |
| Galzener and Jull (1946) | thiouracil | positive | |
| Dvoskin (1947) | thiouracil | positive | |
| Mixner and Upp (1947) | thiouracil | positive | |
| Kleinpeter and Mixner (1947) | thiouracil | positive | |
| Andrews and Bohren (1947) | thiouracil | positive | |
| | **The Turkey** | | |
| Reineke et al. (1946) | thiouracil | positive | |

## Measurement of Thyroid Secretion Rate

With the knowledge of normal thyroid physiology and the manner in which it is affected by goitrogen administration, we can now describe the measurement of thyroid secretion rate.

Goitrogen Administration. The administered goitrogens prevent the formation of thyroxine by the thyroid gland and as a result the amount of circulating thyroid hormone is lowered. This lowered amount of circulating thyroid hormone stimulates the secretion of additional amounts of thyrotrophic hormone by the pituitary gland.

The thyroid gland responds to the additional amounts of thyrotrophic hormone with hypertrophy and hyperplasia of the follicular epithelial cells and a simultaneous increase in size and weight. Although the gland appears to be secreting large amounts of thyroid hormone, Webster et al., (1928) in the rabbit and Mackenzie and Mackenzie (1943), Astwood et al., (1943), and Reineke et al., (1945) in the rat have shown that following goitrogen feeding the basal energy metabolism is lowered.

Simultaneous Administration of Goitrogens and Thyroxine. By injecting thyroxine into animals fed simultaneously with a goitrogen, the amount of thyrotrophic hormone secreted by the pituitary gland is reduced. As a result, normal thyroid histology is maintained, (Purves, 1943, Mackenzie and Mackenzie, 1943, and Higgins and Joneson, 1946a, 1946b), and thyroid weights can be kept within control values, (Dempsey and Astwood, 1943, Mackenzie and Mackenzie, 1943, and Mixner et al., 1944).

In addition, the basal energy metabolism may be maintained within normal limits through the simultaneous administration of thyroxine and goitrogens, (Dempsey and Astwood, 1943, and Whitehead, 1943). Reineke, Mixner, and Turner (1945) showed that the amount of thyroxine required to maintain normal thyroid weight in rats is the same amount that is required to maintain normal carbon dioxide production.

Goitrogens do not interfere with effect of thyroxine injections on oxygen consumption in rats, (Whitehead, 1943), or with the effect of thyroxine on the pituitary cells in thyroidectomized rats, (Griesbach and Purves, 1945).

By varying the amount of thyroxine injected into goitrogen-fed animals the amount of thyrotrophic hormone released from the pituitary gland may be regulated.

Large amounts of injected thyroxine restrict the secretion and release of thyrotrophic hormone and as a result the thyroid glands are small or atrophic. By supplying very small quantities of thyroxine simultaneously with goitrogen feeding, large amounts of thyrotrophic hormone are secreted into the blood stream and the thyroid glands become enlarged.

The amount of thyroid hormone required to maintain normal thyroid size when administered simultaneously with a goitrogen represents the amount of hormone that would normally be present in maintaining a normal pituitary-thyroid balance. This required amount of thyroxine, then, represents the thyroid secretion rate of the animal under the stated conditions of an experiment.

## EXPERIMENTAL

### Animals

Four strains of mice were used in these studies: the Schwing, Rockland, C3H, and yellow.

The Schwing mice, purchased from Ed. Schwing of Harrison, Ohio, are an albino strain which has been maintained for 40 years without the introduction of any new stock.

The Rockland mice, an all-purpose albino strain developed in 1931 by Rockland Farms, New City, New York, were purchased from Arthur Sutter of Springfield, Missouri.

The C3H strain, closely inbred and mammary-tumor susceptible, were purchased from the Roscoe B. Jackson Memorial Laboratory, Bar Harbour, Maine.

The yellow mice used in this study are the result of a cross between yellow males originally purchased from the Roscoe B. Jackson Memorial Laboratory and Rockland females.

Animals were kept at 78 to 82° Fahrenheit with the exception of one experiment in which the effects of different temperatures on thyroid secretion rate were observed.

## Feed

At the beginning of these studies, the feed used was mixed at the University of Missouri Dairy Barn and consisted of the following:

| Ingredient | Parts by weight |
|---|---|
| yellow corn meal | 45.00 |
| shorts | 15.00 |
| soybean oil meal | 15.00 |
| alfalfa meal | 10.00 |
| meat scraps | 7.00 |
| bran | 5.00 |
| bone meal | 0.50 |
| common salt | 1.00 |
| codliver oil (400 A.O.A.C. units vitamin A per gram) | 0.25 |

Although this feed was suitable for growth, pregnancy, and lactation in mice, it was discontinued because post-war feed shortages necessitated frequent substitutions which were not adequate for growing or lactating mice.

For the remainder of the studies, Purina Laboratory Chow was used. This proved satisfactory for these experiments.

Feed and water were supplied ad libitum. This is especially important when working with mice because the animals resort to cannibalism if feed is restricted.

## Hormones

Thyroxine. Source: D,L-crystalline thyroxine synthesized by the Schering Corporation was used in these experiments.

Preparation: The thyroxine was prepared for injection first by dissolving it in 0.1 normal sodium hydroxide and then by diluting the solution to approximately the required volume with distilled water. One-tenth normal hydrochloric acid was then added until the mono-sodium salt of thyroxine began to precipitate out. The resultant mixture was diluted to volume with distilled water and stored as a stock solution at four degrees Centigrade.

When needed for injection, required amounts of solution were removed from the stock solution and several drops of 0.1 normal sodium hydroxide were added to dissolve the mono-sodium salt.

Administration: Mice were weighed daily and injected according to weight. The injection volume was kept constant at 0.1 of a cubic centimeter by varying the strength of the solutions. Each solution was prepared for a given dosage to cover a five gram range in the body weight of the animals. Injections were made subcutaneously in the mid-dorsal region.

Thyroprotein. Source: Thyroprotein, an iodinated casein manufactured under the trade name of "Protamone," was obtained from the Cerophyl Laboratories of Kansas City, Missouri.

Composition: The sample of thyroprotein contained 2.91 per cent thyroxine as determined by the method of Reineke, Turner, Kohler, Hoover, and Beezley (1945). Harrington and Salter (1930) have shown that thyroxine exists in the thyroid in the levo-form. Since Reineke and Turner (1943) have recovered L-thyroxine from thyroprotein and also have shown that the L-thyroxine present in thyroprotein can account for the thyroxine-activity of thyroprotein, it has been assumed that the 2.91 per cent thyroxine content of the thyroprotein used in these experiments has been in the levo-form. Reineke and Turner (1945) also have shown that L-thyroxine has twice the physiological potency of racemic D,L-thyroxine.

Administration: Thyroprotein was administered orally by mixing it in ground feed. It was first mixed with a small amount of feed and this feed was then successively incorporated into larger batches of feed until the desired weight was reached.

Dianisylhexene (Dimethyl ether of diethylstilbestrol). Source: Dianisylhexene was obtained from Merck and Company, Rahway, New Jersey.

Administration: The dianisylhexene used in these experiments was fed to the animals. It first was dissolved in warm soybean oil and then the oil was incorporated into the feed.

## Goitrogen

Thiouracil. Source: The thiouracil used in this work was prepared by Lederle Laboratories, Pearl River, New York, under the trade name of "Deravet." It is 2-thio-6-oxypyrimidine.

Administration: All of the thiouracil given to the mice was mixed into the feed in a manner similar to the mixing of thyroprotein.

## The Effect of Administering Thiouracil on the Thyroid Gland of the Mouse

The measurement of thyroid secretion rate by observing the amount of thyroid hormone required to maintain normal thyroid size when the animal is simultaneously receiving a goitrogen, is dependent upon the ability of the goitrogen to cause thyroid enlargement.

Although Dalton et al., (1946) noted thyroid hypertrophy and hyperplasia following the feeding of thiouracil to C3H mice, Waldo (personal communication) observed little thyroid enlargement following thiouracil administration to young dba and C57 strain mice. Only moderate hyperplasia was obtained in mice administered methyl-thiouracil by Freiesleben et al., (1945).

In view of these conflicting results it was necessary, then, to determine the effects of feeding thiouracil on the weights of the thyroid glands of the mice that we expected to use in these experiments.

The work of Pipes and Turner (1946a, 1946b) indicates that in the goat and fowl, a given dosage of thiouracil is rapidly eliminated from the blood. If chickens were fed at intervals during the day, however, a fairly high and uniform blood level of thiouracil was maintained. Mice eat often during both the day and night, and it is believed that with the incorporation of thiouracil into the regular ration a fairly high level of thiouracil was maintained in the blood.

Feeding 0.05 per cent thiouracil did not increase thyroid size significantly and feeding 0.1 per cent gave inconsistent results, (Table 2). The feeding of 0.2 per cent thiouracil in the feed, however, caused a highly significant increase in thyroid size. In the following work, the feeding of 0.2 per cent thiouracil was used in determining thyroid secretion rates.

Food consumption was decreased by feeding thiouracil. That decreased appetite was due to a lowered metabolism and not to a dislike for the taste of thiouracil is shown by two factors. First, decline in appetite was slow. Usually if animals do not like a given feed, they start to refuse it immediately. Metabolism, on the other hand, declines slowly following goitrogen administration because although the goitrogen prevents the formation of any additional thyroid hormone, it does not interfere with the release or action of any preformed hormone.

Secondly, it will be shown in a later section that appetite may be maintained at control levels by the simultaneous administration of thiouracil and thyroxine. The thyroxine must be administered in amounts equal to the animal's own thyroid secretion rate.

### The Effect of Administering Thiouracil on Growth

Inasmuch as thiouracil is used in the determination of the thyroid secretion rate, it seemed advisable to present the results of experiments showing the effects of thiouracil and other goitrogens on growth.

Review of Literature. Two general conclusions may be drawn from the experimental data reported concerning the effects of the goitrogens on growth. They are, first, that the younger the animal the greater the effect of goitrogens on retardation of growth, and secondly, toward the end of the growth phase the dominant action of the goitrogens is to accelerate the deposition of fat.

In view of these conclusions, the reported experimental data are presented according to the effects of the goitrogens on growth during fetal life, the suckling period, the period immediately following weaning, and later stages.

Fetal life. In the pregnant mouse, thiouracil passes through the placenta and alters fetal thyroid histology beginning the sixteenth day, (Kauffman et al., unpublished data).

In the goat, Schultze and Turner (1945) found that administering thiouracil or thiourea to pregnant females had little effect on the thyroid glands of the fetuses before mid-term, but following that time there was considerable fetal thyroid enlargement. Although thiouracil passes through the rat placenta, (Monroe and Turner, 1946), there is no permanent damage to the thyroid tissue of the young as shown by Freiesleben and Kjerulf-Jensen (1946) who maintained normal weight gains in young rats which were removed from their thiouracil-fed dams at parturition and were nursed by normal foster-mothers. Goldsmith et al., (1945) fed thiourea to pregnant rats from one to 15 days pre-partum. Although the thyroids of the young were hyperplastic, there was no deviation from the normal in the number of young per litter, litter weights, or their appearance.

Suckling young. When suckling rats received thiouracil through the milk, (Hughes, 1944, Freiesleben and Kjerulf-Jensen, 1946), or thiourea, (Goldsmith et al., 1945), their growth was retarded. Hughes (1944) observed that younger suckling rats are retarded to a greater extent than are older rats in their growth rates.

Weaned mammals and young chickens. Mackenzie et al., (1941) reported that in weaned rats fed sulfadiazine, growth was not affected until after four weeks of treatment although hyperplastic thyroids had been observed earlier. Astwood et al., (1943) reported similar results.

Astwood (1945) observed that a small dose of thiouracil, 0.01 per cent, in the feed of rats from weaning time for a period of 9.5 months increased growth over controls. Not only was this an increase in body weight, but there was also a proportionate gain in skeletal dimensions as well. Apparently the thyroid secretion rate had been slightly reduced and this may be conducive to growth. There are other indications that a slight reduction in thyroid secretion may be beneficial to the rat. Monroe and Turner (unpublished data) have observed increased growth in thiouracil-fed rats which were receiving in the feed smaller amounts of thyroxine than are secreted by their own thyroids. Leonard and Reece (1941) and Smithcors and Leonard (1942) have found that thyroidectomy in the rat stimulated mammary gland growth.

The work of Palmer et al., (1946), also aids in the understanding of the relation of the thyroid to growth in the rat. By breeding selection these authors have developed two strains of rats of which one, a "high efficiency" strain, utilizes food more efficiently during growth than does the other, a "low efficiency" strain. Observations have shown that the "low efficiency" strain has a higher basal metabolic rate and stores less fat and protein than the "high efficiency" strain. The authors concluded from this that the "high efficiency" strain rats may normally produce less thyroxine than the "low efficiency" rats. This supposition is in agreement with the theory that a low thyroid secretion rate is beneficial to the growing rat.

TABLE 2 -- EFFECT OF FEEDING THIOURACIL ON THYROID WEIGHTS AND FEED CONSUMPTION OF MATURE SCHWING FEMALE MICE

| Thiouracil in Feed | Number of Animals | Average Body Weight | Average Thyroid Weight | Average Thyroid Weight, Milligrams per 100 grams Body Weight | Average Amount of Feed Consumed per Animal per day |
|---|---|---|---|---|---|
| per cent | | grams | milligrams | | grams |
| none | 10 | 17.1 ± 0.4520 | 1.5 ± 0.2165 | 8.5 ± 0.3586 | 3.1 |
| 0.05 | 10 | 17.1 ± 0.4530 | 2.1 ± 0.1227 | 12.5 ± 0.5992 | 2.5 |
| 0.10 | 10 | 17.9 ± 0.2900 | 4.1 ± 0.4017 | 22.9 ± 1.9171 | 2.5 |
| none | 10 | 16.2 ± 0.6900 | 2.2 ± 0.2548 | 13.7 ± 1.2844 | -- |
| 0.05 | 10 | 18.8 ± 0.7434 | 2.8 ± 0.2965 | 14.8 ± 1.4126 | -- |
| 0.10 | 10 | 19.7 ± 1.1564 | 3.7 ± 0.2629 | 19.0 ± 2.4856 | -- |
| 0.20 | 10 | 19.1 ± 0.9921 | 5.2 ± 0.8565 | 27.3 ± 3.3781 | -- |

Means Given with Standard Errors

Palmer et al., (1946) also administered desiccated thyroid in equal amounts to the "high efficiency" and "low efficiency" strains of rats during growth and found that growth was retarded in both strains. Growth was retarded to a greater extent in the "high efficiency" strain which again indicates that this is the strain with the lower thyroid secretion rate since the greater the extent to which a growing rat is stimulated above its own thyroid secretion rate, the more detrimental are the effects on growth.

Higgins and Joneson (1946b) noted that promizole depressed growth in the rat.

In the week-old chick, Astwood et al,, (1944) noted that large dosages of thiouracil markedly retarded growth and Glazener and Jull (1946) found that thiouracil administration inhibited growth and feed utilization in chicks.

Older animals. Leblond and Hoff (1944) observed that both thiouracil and sulfathiazole retarded growth in young male rats but that sulfadiazine did not.

Kempster and Turner (1945) depressed body weight gains in chickens placed on thiouracil at eight weeks of age but failed to depress gains in those started at ten weeks. Chickens were retarded in growth when fed thiouracil between six and 16 weeks of age, (Andrews and Schnetzler, 1946, and Andrews and Bohren, 1947).

Cockerels 16 weeks old fed thiouracil gained more weight after four weeks of thiouracil feeding than did controls, (Mixner et al., 1946). Thiouracil administration had no effect on increasing weight in chickens weighing 2.8 kilograms, (Glazener and Jull, 1946).

McQuillan and Trikojus (1946) slowed the growth rate of guinea pigs by administering thiourea. Feeding propylthiouracil had no effect on the growth of dogs, (Mayer, 1947), although the thyroid glands were hyperplastic.

Andrews et al., (1946, 1947) studied the effects of feeding goitrogens on weight gains in lambs. Lambs weighing 74 pounds were placed on experiment for 84 days. Those receiving thiouracil in dosages ranging from 0.175 to 0.544 grams per day showed no significant changes in weight gains from those of control animals, but lambs receiving 1.148 grams of thiouracil per day showed decreased weight gains. Thiourea feeding in dosages ranging from 0.048 to 0.071 grams per day reduced weight gains.

In swine, Muhrer and Hogan (1945) observed that in paired feeding trials lasting 28 days, 130 pound animals receiving 0.2 per cent thiouracil in the ration made more rapid weight gains than did the controls. Van Der Noot et al., (1947) reported more rapid weight gains in 200 to 230 pound swine fed 0.15 to 0.25 per cent thiouracil in the ration for 38 to 45 days than in controls. McMillan et al., (1946, 1947) gave 0.1 per cent thiouracil in the feed to swine weighing 130 to 160 pounds for 41 days. They observed decreased gains in body weight as compared to controls. In pigs fed 0.05 per cent or 0.1 per cent thiouracil for 28 days, Muhrer et al., (1947) reported that the thiouracil-fed pigs contained three per cent less fat then did the controls.

Beeson, Andrews, and Brown (1946, 1947) fed yearling steers weighing approximately 790 pounds in body weight a ration containing two, four, or six grams of thiouracil daily per steer for 98 days. No decrease in weight gains was observed. Ensminger et al., (1947) concluded that feeding thiouracil may be effective in increasing body gains over controls for short periods of time.

The Effect of Feeding Thiouracil to Mice. Thiouracil fed in the ration was effective in retarding growth in mice. Body growth was retarded to a greater extent during the period immediately following weaning and also by the larger dosages employed. From the longer experimental periods in these studies it appears that the thiouracil-fed animals grow as well as the controls. That this was apparent and not real growth on the part of the thiouracil-fed groups is shown by carcass analyses which showed a group fed thiouracil to be considerably fatter than the control animals.

Thiouracil was mixed in the feed at varying levels and the animals were fed ad libitum. All animals were weighed at weekly intervals and at the end of a given experiment they were sacrificed by exposing them to natural gas. Thyroids were immediately removed, trimmed of excess tissue, and weighed. Trials were run for two, six, ten, and twelve weeks.

Thiouracil fed two weeks. The mice in this experiment were fed thiouracil beginning at the age of three weeks. All of the animals raised in our animal colony are weaned at this time and "weaning time" will be used in the following discussions.

In all of the levels of thiouracil fed, 0.05, 0.1, and 0.2 per cent, growth was retarded. Thyroid glands were enlarged although there was a considerable variation in any group as evidenced by the values of the standard errors, (Table 3).

Males and females were grouped together because the groups were small and also because there were no observed sex differences.

Thiouracil fed six weeks. It was observed that 0.2, 0.4, and one per cent thiouracil in the feed retarded growth in Schwing female mice fed for six weeks following weaning, (Figure 1). The one per cent group is not shown beyond the fourth week because the few remaining animals were the smaller animals of the group to begin with, and although they did grow slightly, their average weights shown past the fourth week would create a false impression that feeding of one per cent thiouracil had reduced body weight below the average weaning weight of the entire experimental group.

These animals were not sacrificed inasmuch as they were being raised to study the effects of thiouracil fed during early growth on the ability of the animals to breed.

Thiouracil fed ten and twelve weeks. Thiouracil feeding may have retarded growth in these experiments although direct proof is lacking, (Figures 2 and 3). To be sure, at the end of the experiments there were no statistically significant differences in body weight between thiouracil-fed and control groups, (Table 4). The thiouracil-fed females, however, contained 3.4 per cent more fat than did the controls on carcass analyses, and in the sense that true growth is represented by nitrogeneous gain, the rate of true growth may have been slowed. That there were no significant differences between the tibia lengths of the thiouracil-fed and control animals at the end of the experiment does not exclude the possibility of some growth retardation during the experiment.

## THE THYROID SECRETION RATE DURING GROWTH AND ITS IMPORTANCE IN REGARD TO GROWTH STIMULATION

Thyroid function during growth can be quantitatively described in terms of the animal's own thyroid secretion rate. Whether it is advantageous to decrease or increase the amount of available thyroxine in order

TABLE 3 -- EFFECT ON GROWTH OF THIOURACIL FED 2 WEEKS FOLLOWING WEANING. SCHWING STRAIN, MALE AND FEMALE MICE

| Thiouracil in Feed | Number of Animals | Average Initial Body Weight | Average Final Body Weight | Average Thyroid Weight | Average Thyroid Weight, Milligrams per 100 Grams Body Weight | Average Increase in Body Weight | Group Mortality |
|---|---|---|---|---|---|---|---|
| per cent | | grams | grams | milligrams | | per cent | per cent |
| none | 10 | 8.0 ± 0.2946 | 15.5 ± 0.6732 | -- | -- | 93 | 0 |
| none | 11 | 8.1 ± 0.9171 | 15.6 ± 1.9010 | 2.1 ± 0.1318 | 13.3 ± 1.0917 | 93 | 0 |
| none | 9 | 9.6 ± 0.3439 | 17.2 ± 0.6062 | -- | -- | 79 | 0 |
| 0.05 | 6 | 8.9 ± 0.4030 | 14.3 ± 0.9170 | 6.6 ± 0.7353 | 46.1 ± 4.0661 | 61 | 0 |
| 0.10 | 11 | 11.1 ± 0.4840 | 15.0 ± 0.5988 | 9.5 ± 0.1820 | 62.8 ± 4.5665 | 35 | 0 |
| 0.20 | 9 | 8.0 ± 0.4177 | 12.1 ± 0.3334 | 10.5 ± 1.2847 | 86.8 ± 10.2331 | 51 | 0 |
| 0.20 | 9 | 6.9 ± 0.4931 | 11.9 ± 0.6306 | 8.1 ± 0.9267 | 68.1 ± 6.5777 | 72 | 18 |
| 0.20 | 8 | 6.2 ± 0.3687 | 10.8 ± 0.4807 | 9.7 ± 0.7395 | 89.8 ± 5.2611 | 74 | 0 |

Means Given with Standard Errors

TABLE 4 -- THE EFFECTS OF FEEDING 0.1 PER CENT THIOURACIL TO GROWING ROCKLAND MICE

| Group | Number of Animals | Weeks on Experiment | Average Initial Body Weight | Average Final Body Weight | Average Thyroid Weight | Average Thyroid Weight, Milligrams per 100 Grams Body Weight | Length of Right Tibia | Percentage of Fat, Dry Weight Basis |
|---|---|---|---|---|---|---|---|---|
| | | | grams | grams | milligrams | | centimeters | |
| | | | | Males | | | | |
| Control | 10 | 10 | 19.1 ± 0.1419 | 36.0 ± 1.3504 | 3.6 ± 0.4498 | 9.9 ± 0.9902 | 1.81 ± 0.0228 | -- |
| Thiouracil | 10 | 10 | 19.0 ± 0.3251 | 35.3 ± 0.8904 | 24.6 ± 2.9189 | 69.4 ± 7.4641 | 1.84 ± 0.1118 | -- |
| | | | | Females | | | | |
| Control | 10 | 12 | 18.0 ± 0.5661 | 31.3 ± 0.8132 | 4.1 ± 0.2848 | 13.2 ± 0.9284 | 1.86 ± 0.0362 | 34.39 |
| Thiouracil | 10 | 12 | 16.8 ± 0.5583 | 29.2 ± 1.0861 | 24.2 ± 1.3603 | 82.7 ± 3.7372 | 1.83 ± 0.0194 | 37.70 |

Means Given with Standard Errors

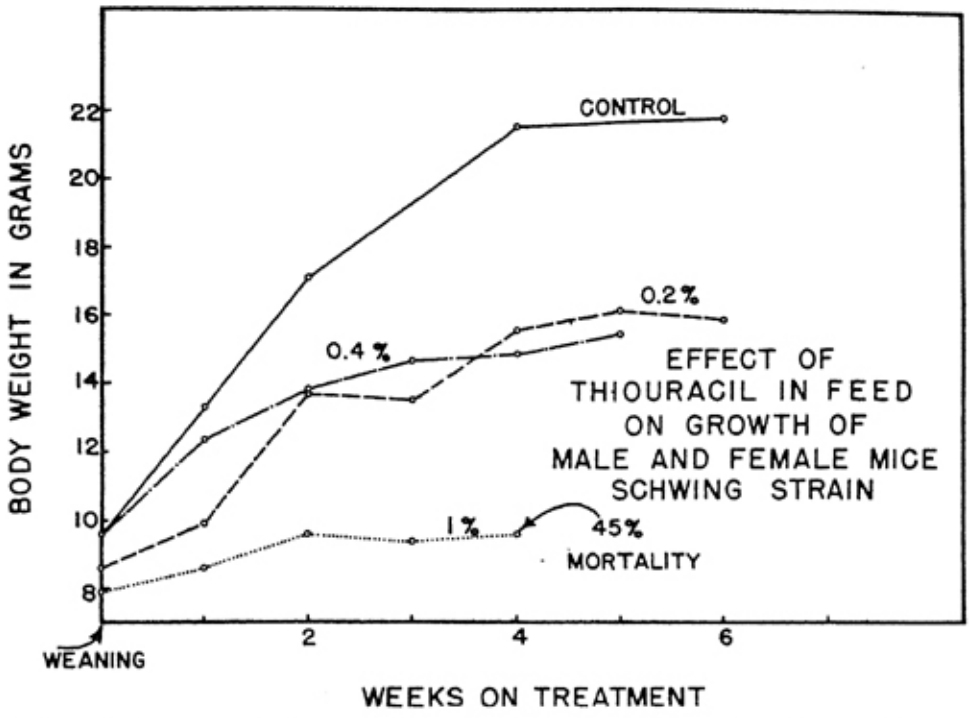


**Fig. 1. Effect of thiouracil in feed on growth of male and female mice, Schwing strain.**

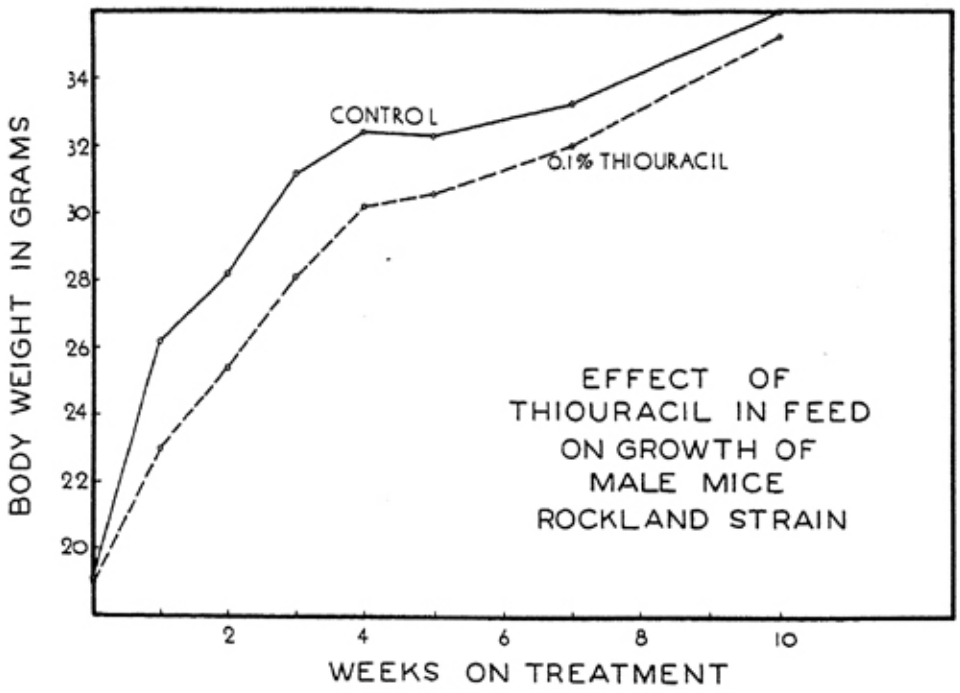


**Fig. 2. Effect of thiouracil in feed on growth of male mice, Rockland strain.**

to stimulate growth appears to be a question of species difference. Thus in the rat, a slight degree of hypothyroidism is beneficial to growth, (Astwood, 1945, Monroe and Turner, unpublished data), whereas in the mouse, Robertson 1928) and Koger and Turner (1943) have observed that mild hyperthyroidism stimulates growth over that of control animals.

Since the thyroid secretion rate per 100 grams body weight declines with age in the chicken, (Schultze and Turner, 1945), and in the rat, (Monroe and Turner, 1946), the question arises as to how the animal

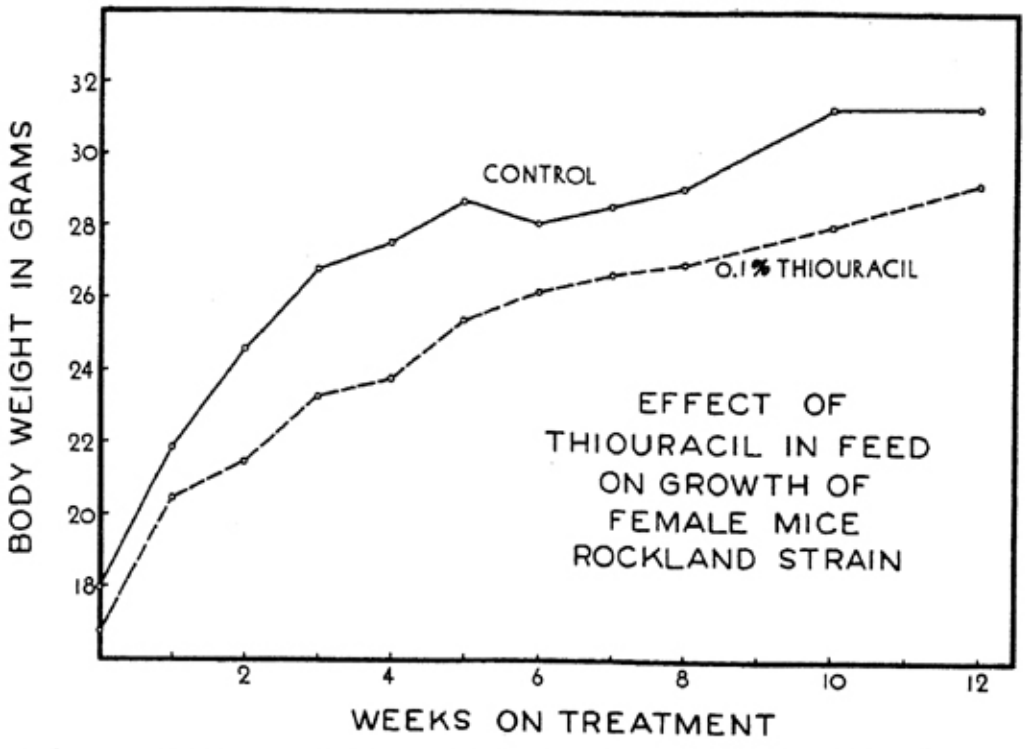


**Fig. 3. Effect of thiouracil in feed on growth of female mice, Rockland strain.**

should be stimulated in order to grow at a more rapid rate than control animals. Should a constant dosage of thyroxine be administered throughout the growth period, or should the dosage level decline inasmuch as the animal's own thyroid secretion rate would be declining?

Also, if dosage levels were decreased as the animals reached mature size, should they be decreased at a ratio of the declining thyroid secretion rate?

These problems, along with a study of the thyroid secretion rate during growth, will be discussed in this section.

## Preliminary

In a preliminary experiment, young Schwing mice, three to five weeks of age, were fed thiouracil and injected with thyroxine simultaneously. The mice were not segregated according to sex because of the small numbers in the groups and because no differences were observed in the responses to treatment by the males and females. With the environmental temperature averaging 80° Fahrenheit, the young mice secreted the equivalent of 9.2 micrograms of D,L-thyroxine per 100 grams body weight per day, (Table 5). Inasmuch as these animals were growing, they served to illustrate an interesting point in connection with the thyroid secretion rate assay used in this work. With the animal's own thyroid secretion inactivated by the feeding of thiouracil, the only source of thyroid hormone would be the amount that was being injected. It would follow, therefore, that the amount of thyroxine required to maintain the thyroid gland at normal size would also be enough to cause normal growth gains. This was found to be the case. The animals which received ten micrograms of D,L-thyroxine per 100 grams body weight gained 85 per cent of their initial body weight as compared to a gain of 93 per cent by the controls. The differences in the body weights at the end of the experiment between the group injected with ten micrograms of thyroxine

TABLE 5 -- THYROID SECRETION RATE FOLLOWING WEANING, SCHWING STRAIN OF MICE

| Thiouracil in Feed | D,L-Throxine Injected per 100 grams Body Weight per day | Number of Animals | Average Initial Body Weight | Average Final Body Weight | Average Thyroid Weight | Average Thyroid Weight, Milligrams per 100 Grams Body Weight | Average Increase in Body Weight |
|---|---|---|---|---|---|---|---|
| per cent | micrograms | | grams | grams | milligrams | | per cent |
| none | none | 11 | 8.1 ± 0.9171 | 15.6 ± 1.9010 | 2.1 ± 0.1318 | 13.3 ± 1.0917 | 93 |
| 0.2 | none | 9 | 8.0 ± 0.4177 | 12.1 ± 0.3334 | 10.5 ± 1.2847 | 86.8 ± 10.2331 | 51 |
| 0.2 | 2.5 | 9 | 7.3 ± 0.5421 | 11.2 ± 0.3644 | 8.6 ± 0.9131 | 77.9 ± 4.7648 | 53 |
| 0.2 | 5.0 | 11 | 8.9 ± 0.3766 | 15.3 ± 0.4313 | 6.1 ± 0.9649 | 39.5 ± 6.3329 | 72 |
| 0.2 | 10.0 | 8 | 7.7 ± 0.4980 | 14.8 ± 0.0935 | 1.2 ± 0.2943 | 8.1 ± 1.2823 | 85 |

Means Given with Standard Errors

| | |
|---|---|
| Average body weight in grams | 14.8 |
| Micrograms D,L-thyroxine secreted per mouse per day | 9.2 |
| Micrograms D,L-thyroxine secreted per 100 grams body weight per day - | 1.4 |

TABLE 6 -- EFFECT OF SIMULTANEOUS ADMINISTRATION OF THYROXINE AND THIOURACIL ON GROWTH OF ROCKLAND FEMALE MICE

| Thiouracil in Feed | D,L-Thyroxine Injected per 100 Grams Body Weight per Day | Number of Animals | Average Initial Body Weight | Average Final Body Weight | Average Thyroid Weight | Average Thyroid Weight, Milligrams per 100 grams Body Weight | Average Length of Right Tibia | Mortality |
|---|---|---|---|---|---|---|---|---|
| per cent | micrograms | | grams | grams | milligrams | | centimeter | per cent |
| none | none | 10 | 18.0 ± 0.5661 | 31.3 ± 0.8132 | 4.1 ± 0.2848 | 13.2 ± 0.9284 | 1.86 ± 0.0141 | 0 |
| 0.1 | none | 10 | 16.8 ± 0.5583 | 29.2 ± 1.0861 | 24.2 ± 1.3603 | 82.7 ± 3.7372 | 1.83 ± 0.0194 | 0 |
| 0.1 | 4 | 20 | 18.2 ± 0.5167 | 29.9 ± 0.9935 | 9.1 ± 1.8629 | 29.2 ± 4.5259 | 1.83 ± 0.0105 | 30 |
| 0.1 | 8 | 20 | 18.5 ± 0.2939 | 30.6 ± 0.6132 | 4.1 ± 0.2959 | 13.5 ± 0.9123 | 1.83 ± 0.0122 | 15 |
| 0.1 | 100 | 20 | 18.0 ± 0.2095 | 29.5 ± 0.5883 | 3.3 ± 0.1920 | 11.2 ± 0.7313 | 1.84 ± 0.0004 | 0 |
| 0.1 | 200 | 20 | 17.6 ± 0.3094 | 26.4 ± 0.4034 | 2.5 ± 0.1173 | 9.6 ± 0.4404 | 1.80 ± 0.0193 | 5 |
| 0.1 | 300 | 20 | 18.6 ± 0.2802 | 27.6 ± 0.6041 | 2.7 ± 0.1383 | 9.8 ± 0.6783 | 1.78 ± 0.0095 | 0 |
| 0.1 | 400 | 18 | 18.3 ± 0.4139 | 29.2 ± 0.9361 | 2.9 ± 0.2014 | 10.0 ± 0.5710 | 1.79 ± 0.0202 | 17 |

Means Given with Standard Errors

and the controls were not significant, according to an analysis of variance. Higgins and Joneson (1946b) could not maintain normal growth in promizole-fed rats receiving amounts of thyroxine which were slightly in excess of that required to maintain normal basal metabolism.

## Stimulation of Growth in Mice

Inasmuch as Schwing mice were no longer available after our preliminary experiment with thyroid secretion rate during growth, we began working with the Rockland strain. The Rocklands are faster growing mice with a heavier mature body weight than the Schwing strain. All animals were studied during the most active period of growth. Although the exact age of the animals was not known, it was estimated that their approximate age was four weeks.

It was of interest, then, to see if growth could be stimulated in a more rapidly growing strain of mice. Along with the thyroxine injections a low level of thiouracil was fed, 0.1 per cent, to confirm our own earlier observations; namely, that if sufficient thyroxine above the normal thyroid secretion rate is administered, the simultaneous administration of thiouracil has no effect on the growth of the animals. The action of the thiouracil is to prevent the synthesis of the thyroid hormone and it does not appear to affect the animal otherwise during the growth process.

### (a) Females

Injecting amounts of thyroxine approximating the normal thyroid secretion rate. It was found that if amounts of thyroxine approximating the normal thyroid secretion rate were injected concurrently with the feeding of thiouracil for a nine week period, growth was maintained at a normal rate, (Figure 4). It was observed that although the average thyroid weight of the eight microgram group was similar to that of the controls, the four microgram group had thyroids larger than the control thyroids, (Table 6). This may be explained by assuming that at the beginning of the experiment the animals were secreting more than four micrograms of thyroxine per 100 grams body weight per day. Supplying only four micrograms concurrently with thiouracil, then, would result in thyroid enlargement. Once these thyroids are enlarged, it might take more than the remaining time of the experiment to return them to normal size.

The carcass composition of the mice administered eight micrograms of D,L-thyroxine per 100 grams body weight per day was similar to that of the controls, (Table 7), but the group receiving four micrograms contained less fat and more nitrogen than the controls. No explanation can be offered.

Tibia lengths of the control, four, and eight microgram groups showed no significant variation from each other.

The high mortality of the four and eight microgram groups cannot be explained.

Injecting amounts of thyroxine above the normal thyroid secretion rate. Injecting 100 and 300 micrograms of D,L-thyroxine per 100 grams body weight per day for eight weeks stimulated growth rate above normal during the first part of the experiment. Injecting 200 micrograms for ten weeks and 400 micrograms for 11 weeks did not stimulate growth above normal.

TABLE 7 -- COMPOSITE CARCASS ANALYSES OF MICE, DRY WEIGHT BASIS

| Thiouracil in Feed per cent | D,L-Thyroxine Injected per 100 Grams Body Weight per Day micrograms | Number of Animals | Time on Experiment weeks | Fat per cent | Nitrogen per cent | Ash per cent | Phosphorus per cent | Calcium per cent |
|---|---|---|---|---|---|---|---|---|
| none | none | 10 | 12 | 34.39 | 8.10 | 10.31 | 1.90 | 2.88 |
| 0.1 | none | 10 | 12 | 37.70 | 7.96 | 8.45 | 1.66 | 2.25 |
| 0.1 | 4 | 20 | 9 | 30.65 | 8.50 | 11.49 | 2.14 | 3.26 |
| 0.1 | 8 | 20 | 9 | 34.75 | 8.22 | 10.05 | 1.88 | 2.63 |
| 0.1 | 100 | 20 | 8 | 34.76 | 8.28 | 10.06 | 1.85 | 2.67 |
| 0.1 | 200 | 20 | 10 | 33.78 | 8.41 | 9.74 | 1.87 | 2.67 |
| 0.1 | 300 | 20 | 8 | 32.63 | 8.54 | 9.54 | 1.76 | 2.45 |
| 0.1 | 400 | 18 | 11 | 35.38 | 8.15 | 8.48 | 1.55 | 1.93 |

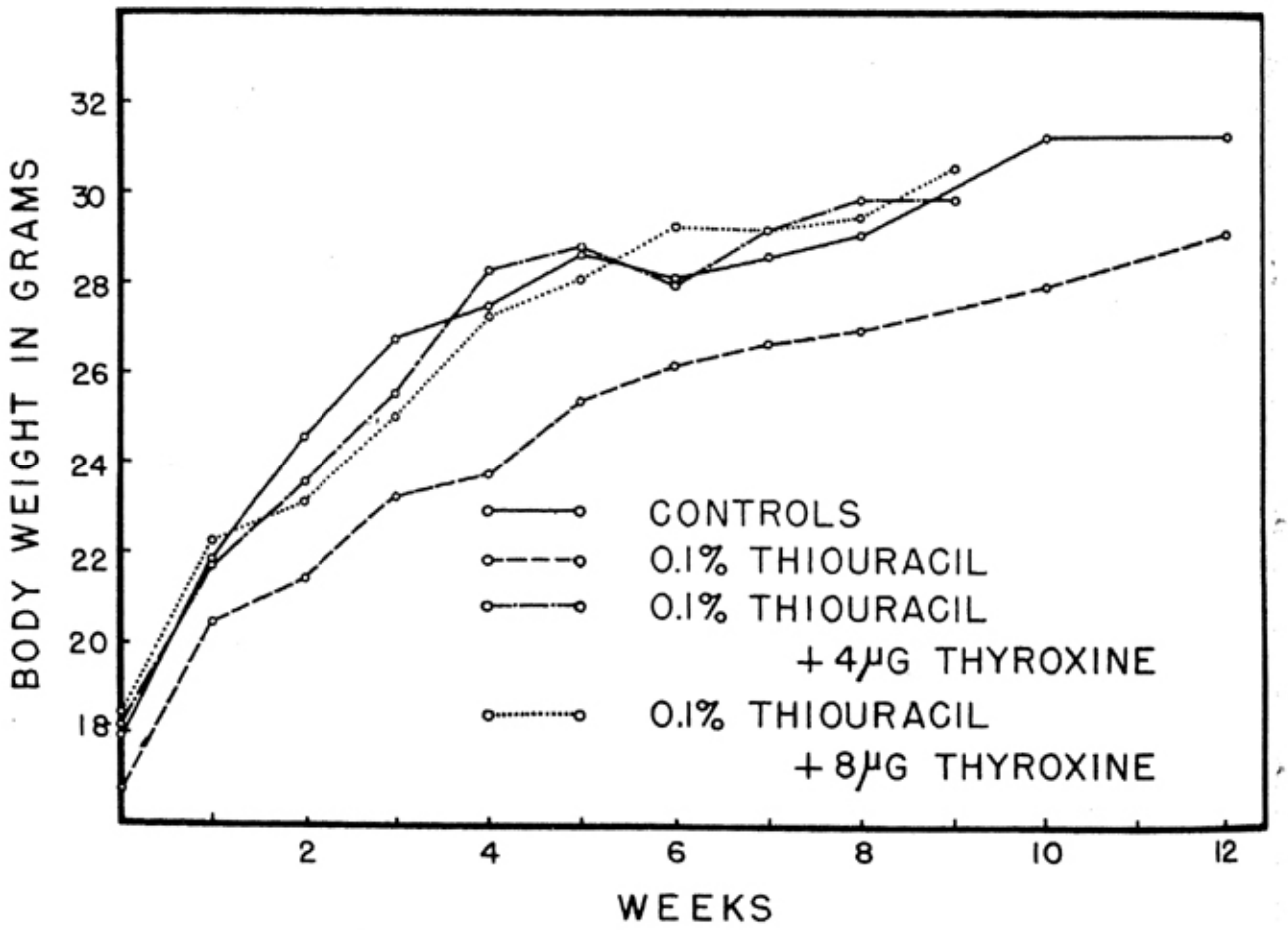


**Fig. 4. Growth in female mice. Effect of injecting thyroxine in amounts approximating normal thyroid secretion rate.**

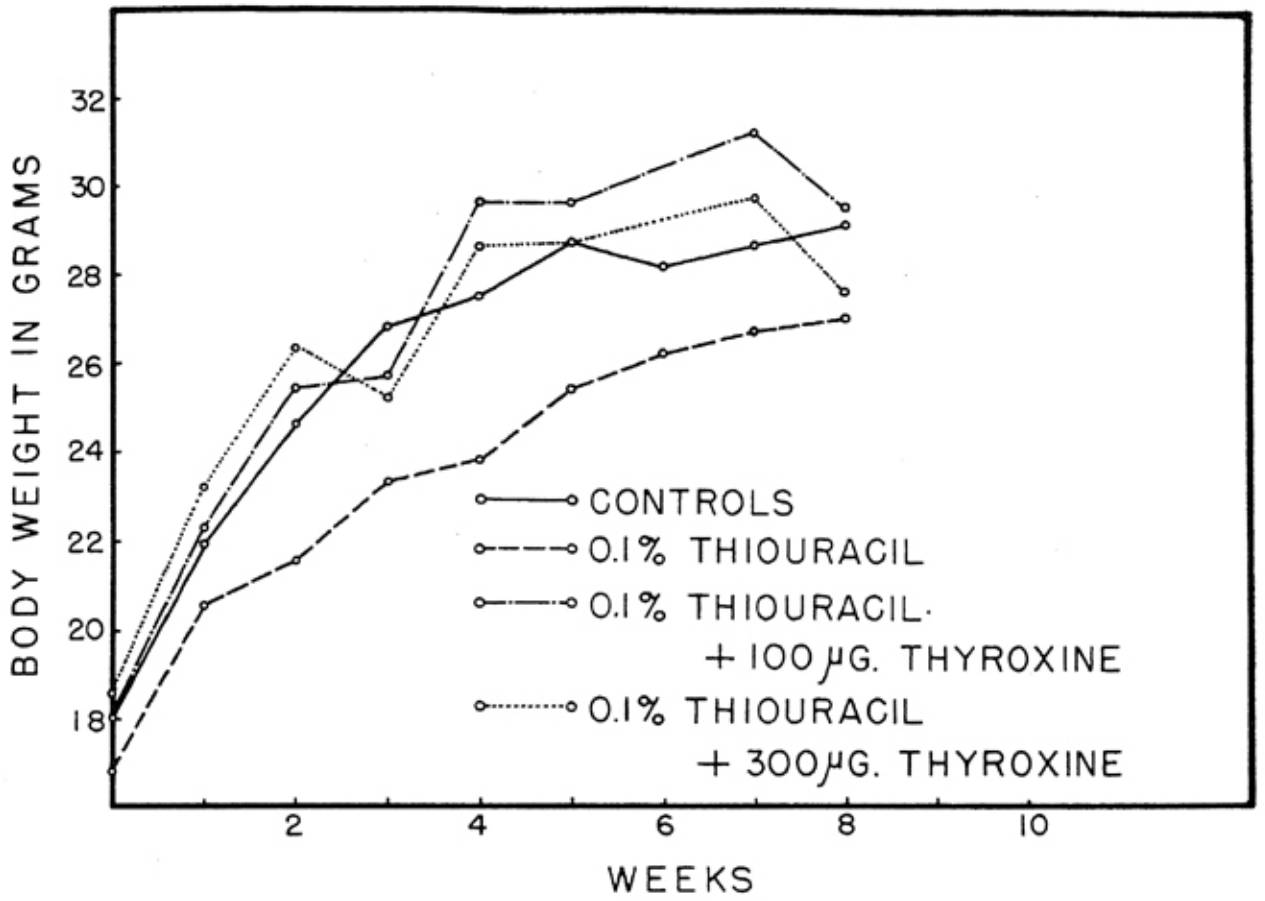


Fig. 5. Growth in female mice. Effect of injecting thyroxine in amounts above normal thyroid secretion rat.

One hundred and three hundred microgram groups. In the 100 microgram group there was no significant increase over control body weights until the end of the fourth week, (Figure 5), at which time the thyroxine-injected group was significantly heavier than the controls. This difference was not maintained very long, however, and the animals appeared in poor condition toward the end of the experiment.

After two weeks of injections, the 300 microgram group had significantly heavier body weights than the control body weights. This advantage was soon lost, however, and although at the end of the experiment the body weights of the injected group were not significantly smaller than those of the controls, the lengths of the tibias were shorter than those of the controls.

The interesting point made here is that 300 micrograms stimulated growth during the early and rapid growth phase whereas 100 micrograms stimulated growth at a later and less rapid growth phase. The suggestion here is that there are optimal levels of thyroid stimulation above the animal's own thyroid secretion rate, but that as the thyroid secretion rate declines in the growing animal, the dosage of thyroxine needed to stimulate growth must be reduced in order to remain within physiological limits.

Two hundred microgram group. During the most active portion of the growth phase there were no significant differences in body weights between the animals receiving 200 micrograms of thyroxine per 100 grams body weight per day and the control animals. By the end of the experiment, however, the 200 microgram group was smaller than the control

TABLE 8 -- EFFECT OF SIMULTANEOUS ADMINISTRATION OF THYROXINE AND THIOURACIL ON GROWTH OF ROCKLAND MALE MICE

| Thiouracil in Feed | D,L-Thyroxine Injected per 100 Grams Body Weight per Day | Number of Animals | Average Initial Body Weight | Average Final Body Weight | Average Thyroid Weight | Average Thyroid Weight, Milligrams per 100 Grams Body Weight | Average Length of Right Tibia | Mortality |
|---|---|---|---|---|---|---|---|---|
| per cent | micrograms | | grams | grams | milligrams | | centimeters | per cent |
| none | none | 10 | 19.1 ± 0.1419 | 36.0 ± 1.3504 | 3.6 ± 0.4498 | 9.9 ± 0.9902 | 1.81 ± 0.0228 | 10 |
| 0.1 | none | 10 | 19.0 ± 0.3251 | 35.3 ± 0.8904 | 24.6 ± 2.9189 | 69.4 ± 7.4641 | 1.84 ± 0.1118 | 10 |
| 0.1 | 8 | 15 | 22.6 ± 0.4817 | 40.1 ± 0.8075 | 3.8 ± 0.2177 | 9.3 ± 0.6705 | 1.87 ± 0.0167 | 27 |
| 0.1 | 100 | 15 | 22.3 ± 0.3852 | 35.2 ± 0.7668 | 4.0 ± 0.1743 | 14.1 ± 0.5836 | 1.84 ± 0.0290 | 27 |

Means Given with Standard Errors

group; the difference in body weights was highly significant statistically, and the right tibias of the group injected with thyroxine were significantly smaller than the controls. That this amount of thyroxine injected for a similar period interferes with reproduction will be shown in a later section.

Four hundred microgram group. Growth rate in this group was irregular and the animals never appeared to be in good condition. Mortality was 17 per cent and probably would have gone much higher if the experiment had been continued any longer. The animals were obviously receiving too much thyroxine.

Judging from the weights of the thyroid glands, (Table 6), the effects of thiouracil in the feed were overcome by all levels of thyroxine injections except for the four microgram group. Other differences among groups appear to be the result of thyroxine injections.

### (b) Males

Eight microgram group. Inasmuch as the group injected with eight micrograms of thyroxine per 100 grams body weight per day for nine weeks was significantly heavier than the control group at the start of the experiment, the only importance attached to their being larger than the controls at the end of the experiment is that they gained as well as the controls, (Table 8, Figure 6).

Thyroid weights were maintained at normal size, and the tibia lengths were significantly greater than the controls, perhaps because the thyroxine-injected animals were larger at the beginning of the experiment.

One hundred microgram group. Growth rate in this group was comparable to that of controls for the first four weeks of injections, (Figure 6). Then a break occurred in the growth curve and rate of growth appeared to be depressed. Body weights, although significantly larger than those of the controls at the beginning of the experiment, did not differ from those of the controls at the end of the experiment. Thyroid weights and tibia lengths were similar to control values.

No further attempts were made to stimulate growth in male mice. They are not satisfactory to work with since they fight continuously unless isolated. By the end of the ten week experiment, many males had large amounts of scar tissue and open wounds were numerous.

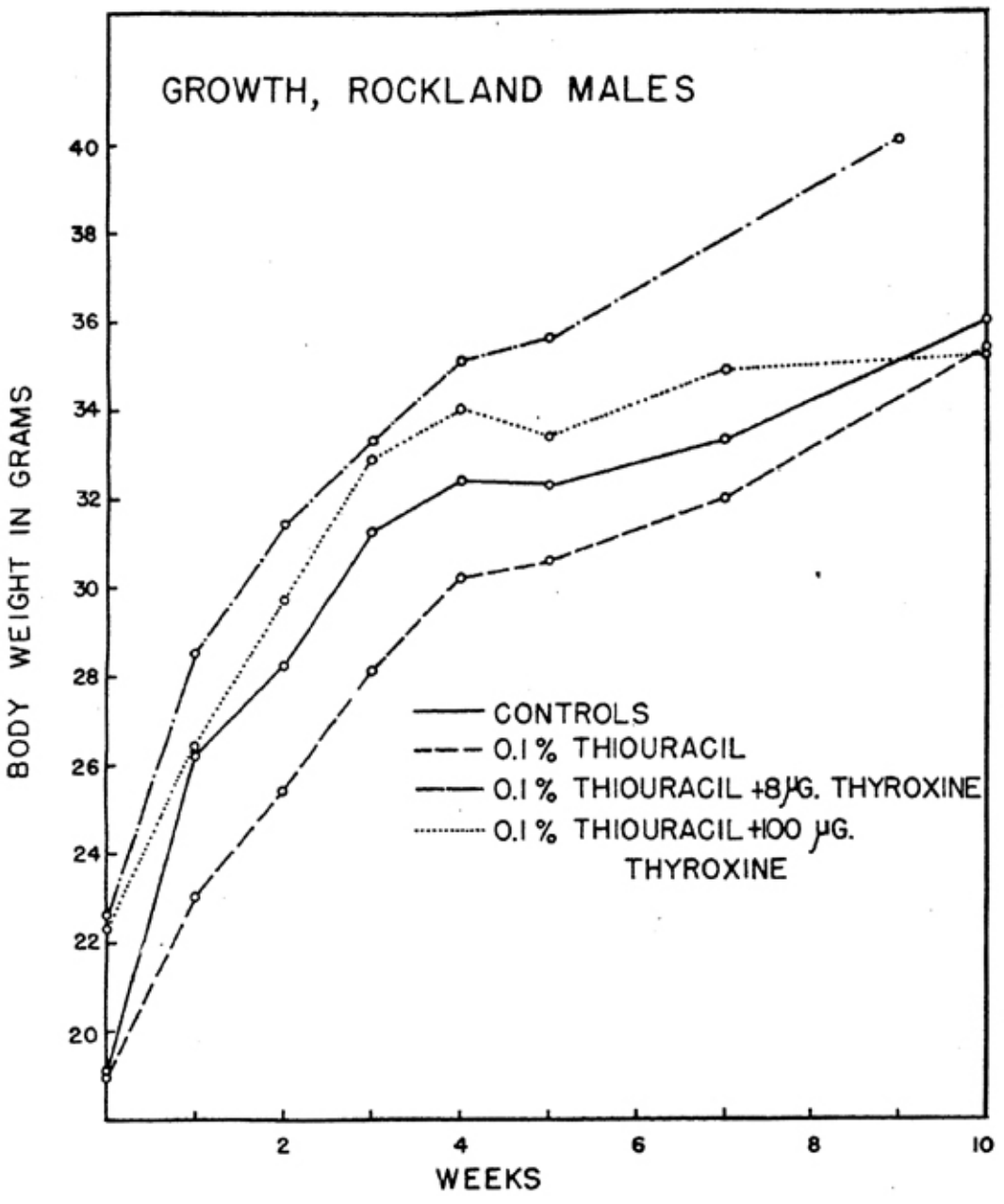


**Fig. 6. Growth in Rockland males.**

## Attempts to Stimulate Growth by Reducing Thyroxine Dosage over a Rapid Phase of Growth in Accordance with a Declining Thyroid Secretion Rate

In an earlier section it was shown that the administration of thyroxine to mice stimulated growth. Further, it was observed that as growth became less rapid, it took smaller amounts of thyroxine to show an increase in gain of thyroxine-injected mice over control mice.

The idea was advanced, then, that with the thyroid secretion rate declining with age, the amount of thyroid hormone required to stimulate growth over normal must necessarily be decreased in order to stay within physiological dosage levels. In this way, an animal could be grown to maturity in a shorter than average time without suffering any adverse effects of overdosage.

This experiment was an attempt to determine an optimal scale of dosage levels with which to stimulate growth and at the same time to study

the concurrent thyroid secretion rate. Two hundred and fifty Rockland female mice of approximately the same age and weight were received from the breeder. Forty experimental and ten control mice were taken at random for the growth trials, and the remaining mice were assayed for their thyroid secretion rates over the growth period. The animals were observed during an eight week period representing their most rapid growth phase.

Dosage levels are given in Table 9. In addition, all but the control animals received 0.1 per cent thiouracil in the feed in order to be consistent with the earlier experiments. When thyroxine injections were terminated in groups "A" and "C," thiouracil was also withdrawn and the animals were placed on control feed.

Thyroid secretion rates were determined at bi-weekly intervals, (Figure 7).

Experimental. At the end of two weeks, all experimental animals were larger than the control animals. Up to this point, all experimental animals had received 300 micrograms of D,L-thyroxine per 100 grams body weight per day, (Figure 8).

By the end of the fourth week, all injected groups again weighed more than controls, although the only group that was significantly heavier was the one in which the thyroxine dosage had been lowered from 300 to 200 micrograms of thyroxine after the second week of the experiment.

After this point, there was a tendency for experimental body weights to approach those of the controls. By the end of eight weeks there were no significant differences between experimental and control body weights.

## The Thyroid Secretion Rate of Growing Yellow Female Mice

Experimental. Growing yellow female mice averaging 23.8 grams in body weight secreted the equivalent of 1.2 micrograms of D,L-thyroxine per day, and 5.0 micrograms per 100 grams body weight per day, (Table 10).

Discussion. Yellow mice are of particular interest because of their inherited ability to deposit large amounts of body fat after reaching sexual maturity.

Not only are yellow mice fat, but Castle (1941) found them to have longer bodies than their black-colored sibs.

Granted that the tendency to deposit fat is carried by the $A^y$ gene which also carries the yellow color, it is of importance to know the manner by which these characteristics are able to express themselves. There has been some evidence presented to show that the ability to deposit fat in yellow mice is mediated by way of the endocrine glands.

Weitze (1940) found that the sensitivity to insulin injection is decreased in yellow mice. Further, blood sugar in yellow mice falls within normal limits following fasting and there is a normal amount of glycogen present in the liver. No ketonuria or glycosuria were observed.

Since yellow mice are fat and have a diminished sensitivity to insulin it may mean that they secrete fairly large amounts of insulin. These large amounts of insulin would tend first to convert glucose to glycogen and then stimulate the formation of fat. If this were true it would imply that a hypersecretion of a pituitary carbohydrate metabolism hormone was present in order to maintain a normal blood sugar level.

TABLE 9 -- TREATMENT OF FEMALE MICE IN DETERMINING OPTIMUM SCALES OF THYROXINE DOSAGE LEVELS FOR STIMULATING GROWTH

| Period Number | Duration of Period | Group "A" (15 mice) | Group "B" (10 mice) | Group "C" (15 mice) |
|---|---|---|---|---|
| | weeks | micrograms of D,L-thyroxine injected per 100 grams body weight per day | | |
| 1 | 2 | 300 | 300 | 300 |
| 2 | 2 | 100 | 300 | 200 |
| 3 | 1 | 10 | 300 | 100 |
| 4 | 2 | 0 | 300 | 10 |
| 5 | 1 | 0 | 300 | 0 |

Weitze also observed that the thyroid glands of yellow mice present a normal histological picture and that yellow mice are as active as as non-yellow mice.

Dickerson and Gowen (1947) reported that yellow mice utilized their food more efficiently than did non-yellow controls.

Yellow mice and their non-yellow sibs exhibit the same early growth curves but at about the weight of 27 grams, non-yellow mice show a sharp break toward leveling off in body weight whereas the yellow mice continue to gain in body weight. This additional gain in body weight is due primarily to the deposition of fat and continues to increase until reaching a weight of about 50 grams (Dickie and Woolley, 1946).

In view of the fact that yellow mice present the same growth curves as their sibs below the weight of 27 grams, it is of interest to note that Rockland female mice, which are growing as rapidly as yellow mice at the weight of about 24 grams, secrete approximately the equivalent of 5.3 micrograms of D,L-thyroxine per 100 grams body weight per day, (Figure 7), as compared to the yellow mice which secrete the equivalent of 5.0 micrograms of D,L-thyroxine per 100 grams body weight per day, (Table 10).

Thus, at a period when there are no apparent differences between the growth curves of Rockland female and yellow female mice, there is no significant difference between their thyroid secretion rates.

## DISCUSSION

It has been shown in these experiments that the thyroid secretion rate of mice declines during the growth period. It was also observed that in order to obtain growth stimulation, the thyroid material which is administered must be given with regard to the animal's own thyroid secretion rate in order to remain within physiological limits.

Evidence is presented to show that growth may be stimulated by reducing the dosage of thyroxine at a time when the animal's own thyroid

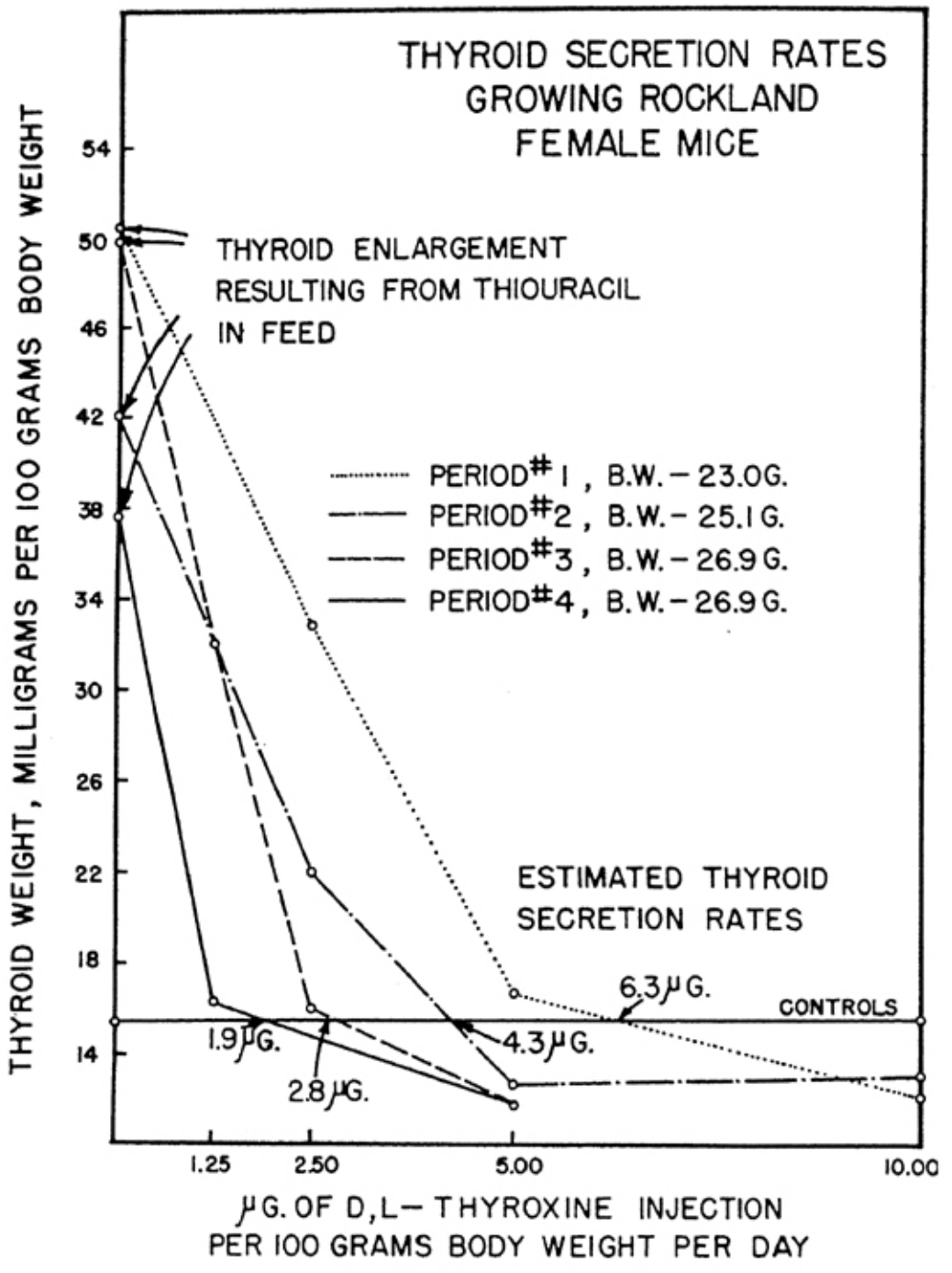


Fig. 7. Thyroid secretion rates in growing Rockland female mice.

secretion rate would be declining. There is some indication that growth is better stimulated by reducing thyroxine dosage during the growth period than it is by maintaining a constant dosage. No constant ratio between a declining thyroid secretion rate and decreasing dosage levels of thyroxine favorable to stimulating growth could be found. Thyroxine administration in the mouse, however, must be given at high levels to stimulate growth at the beginning of the growth period following weaning, and this high level cannot be maintained without producing harmful effects in the animal. It follows, then, that the thyroid secretion rate would act as a good guide for setting up dosages within physiological range, and although only some indication of that may be given in these experiments, there is no evidence to detract from the theory that as the thyroid secretion rate declines during growth, the amount of thyroxine administered to stimulate growth must be decreased.

TABLE 10 -- THYROID SECRETION RATE, GROWING YELLOW MICE

| Thiouracil in Feed per cent | D,L-Thyroxine Injected per 100 Grams Body Weight per Day micrograms | Number of Animals | Average Body Weight grams | Average Thyroid Weight milligrams | Average Thyroid Weight, Milligrams per 100 Grams Body Weight |
|---|---|---|---|---|---|
| none | none | 8 | 24.4 ± 1.4738 | 2.4 ± 0.3538 | 10.1 ± 0.5175 |
| 0.2 | none | 10 | 24.6 ± 1.1457 | 9.6 ± 0.8104 | 35.7 ± 2.6443 |
| 0.2 | 2.5 | 5 | 22.2 ± 2.2781 | 3.1 ± 0.6104 | 14.0 ± 2.9295 |
| 0.2 | 5.0 | 5 | 23.1 ± 0.8639 | 2.3 ± 0.3446 | 10.1 ± 0.4556 |

Means Given with Standard Errors

| | |
|---|---|
| Average body weight in grams | 23.8 |
| Micrograms D,L-thyroxine secreted per mouse per day | 1.2 |
| Micrograms D,L-thyroxine secreted per 100 grams body weight per day | 5.0 |

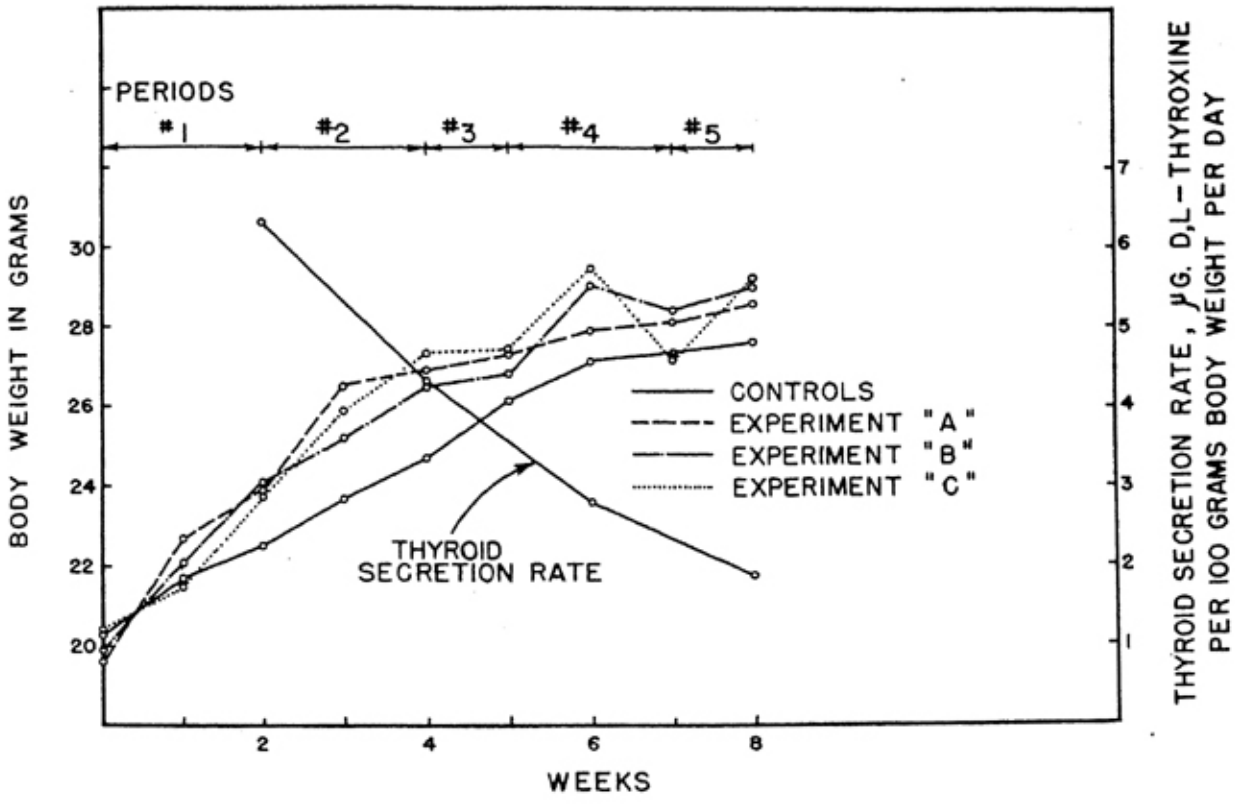


Fig. 8. Treatment for groups shown here is given in Table 9. In addition, this figure shows the concurrent declining thyroid secretion rate.

Within recent years investigators have been obtaining favorable results in stimulating growth by the use of thyroid materials. Their results may be explained in that they have been using dosage levels within the physiological range of response; a range not far from the normal thyroid secretion rate.

In laboratory animals, Robertson (1928) and Koger and Turner (1943) have observed accelerated growth in mice following thyroid administration. Koger and Turner (1943) did not observe changes in growth rates of rats, guinea pigs, or rabbits following thyroid administration. Their results in the rat may be explained by Monroe and Turner (unpublished data) and Astwood (1945) who have observed that a slight degree of hypothyroidism accelerates growth.

In farm animals, where a direct practical application of this work lies, there have been favorable results following the feeding of thyroprotein.

Thyroprotein feeding has accelerated growth in the chick, (Irwin et al., 1943).

In swine, Braude (1946) was not able to secure favorable effects on growth by feeding thyroprotein, possibly because dosage levels were maintained at a constant level during the experiment.

Beeson, Andrews, Witz, and Perry (1947) put pigs weighing 53 pounds on an 84 day feeding trial. In addition to control feed, thyroprotein was mixed into the feed at levels of 0.0044 and 0.0088 per cent. Weight gains were not affected by feeding 0.0044 per cent thyroprotein, but pigs fed 0.0088 per cent thyroprotein gained 26 more pounds and required ten per cent less feed per pound of gain than the control group.

Wallach et al., (1947) observed that during a 79 day post-weaning period, Duroc pigs receiving 1.40 and 2.80 grams of thyroprotein per 100 pounds of feed gained 1.54 pounds daily on an average of 3.40 pounds of feed per pound of gain as compared to controls which gained 1.42 pounds daily on an average of 3.63 pounds of feed per pound of gain. Berkshire pigs receiving 5.6 grams of thyroprotein per 100 pounds of feed made 0.28 pounds more gain daily on 0.94 pounds less feed per pound of gain than their controls.

## THE THYROID SECRETION RATE OF MATURE MICE

The thyroid secretion rate was studied in mature mice of the Schwing, Rockland, and $C_3H$ strains.

### Schwing Strain

The thyroid secretion rate of mature Schwing mice was studied in two experiments.

In the first experiment sexually mature male mice averaging 22.5 grams in body weight secreted the equivalent of 0.5 micrograms of D,L-thyroxine per day, and 2.4 micrograms per 100 grams body weight per day, (Table 11). Sexually mature virgin female mice averaging 19.9 grams in body weight secreted the equivalent of 1.1 micrograms of D,L-thyroxine per day, and 5.5 micrograms per 100 grams body weight per day, (Table 12).

In the second experiment, the males used were sexually mature but younger than those used in the first experiment. They averaged 22.3

TABLE 11 -- THYROID SECRETION RATE, MATURE SCHWING MALE MICE

| Thiouracil in Feed | D,L-Thyroxine Injected per 100 Grams Body Weight per Day | Number of Animals | Average Body Weight | Average Thyroid Weight | Average Thyroid Weight, Milligrams per 100 Grams Body Weight |
|---|---|---|---|---|---|
| per cent | micrograms | | grams | milligrams | |
| none | none | 74 | 20.5 ± 0.4717 | 2.5 ± 0.1243 | 11.6 ± 0.4221 |
| 0.2 | none | 38 | 22.9 ± 0.3928 | 9.7 ± 0.5046 | 42.8 ± 1.7246 |
| 0.2 | 0.125 | 19 | 22.3 ± 0.4511 | 8.5 ± 0.6749 | 37.4 ± 2.5430 |
| 0.2 | 0.250 | 19 | 23.6 ± 0.4941 | 9.0 ± 0.6831 | 37.9 ± 2.5713 |
| 0.2 | 0.500 | 5 | 21.2 ± 1.3838 | 4.1 ± 0.8444 | 19.3 ± 2.7515 |
| 0.2 | 1.000 | 8 | 22.8 ± 1.2937 | 5.9 ± 0.8143 | 25.7 ± 3.2001 |
| 0.2 | 2.000 | 8 | 21.9 ± 0.6541 | 3.4 ± 0.4921 | 15.5 ± 2.0933 |
| 0.2 | 2.500 | 10 | 22.1 ± 0.5040 | 2.3 ± 0.3938 | 10.5 ± 1.8832 |

Means Given with Standard Errors

| | |
|---|---|
| Average body weight in grams | 22.5 |
| Micrograms D,L-thyroxine secreted per mouse per day | 0.5 |
| Micrograms D,L-thyroxine secreted per 100 grams body weight per day | 2.4 |

grams in body weight and secreted the equivalent of 0.7 micrograms of D,L-thyroxine per day and 3.1 micrograms per 100 grams body weight per day, (Table 13). The female mice used in the second experiment were mostly parous and older than the females used in the first experiment. They averaged 24.0 grams in body weight and secreted the equivalent of 0.9 micrograms of D,L-thyroxine per day and 3.6 micrograms per 100 grams body weight per day, (Table 14).

## Rockland Strain

Sexually mature male mice averaging 36.7 grams in body weight secreted the equivalent of 1.0 micrograms of D,L-thyroxine per day, and 2.8 micrograms per 100 grams body weight per day, (Table 13). Sexually mature female mice averaging 32.7 grams in body weight secreted the equivalent of 0.7 micrograms of D,L-thyroxine per day and 2.1 micrograms per 100 grams body weight per day, (Table 14, Figure 9).

## $C_3H$ Strain

Sexually mature female mice averaging 19.6 grams in body weight secreted the equivalent of 0.4 micrograms of D,L-thyroxine per day, and 2.2 micrograms per 100 grams body weight per day, (Table 15).

TABLE 12 -- THYROID SECRETION RATE, MATURE SCHWING FEMALE MICE

| Thiouracil in Feed | D,L-Thyroxine Injected per 100 Grams Body Weight per Day | Number of Animals | Average Body Weight | Average Thyroid Weight | Average Thyroid Weight, Milligrams per 100 Grams Body Weight |
|---|---|---|---|---|---|
| per cent | micrograms | | grams | milligrams | |
| none | none | 24 | 19.9 ± 0.2947 | 2.4 ± 0.1436 | 11.7 ± 0.6206 |
| 0.2 | none | 10 | 20.1 ± 0.6345 | 7.2 ± 0.6041 | 37.3 ± 4.1733 |
| 0.2 | 2.000 | 10 | 19.8 ± 0.6414 | 3.9 ± 0.4817 | 19.4 ± 2.2929 |
| 0.2 | 4.000 | 10 | 19.5 ± 0.3704 | 3.6 ± 0.7247 | 18.5 ± 3.3549 |
| 0.2 | 6.000 | 10 | 20.1 ± 0.4645 | 1.8 ± 0.1929 | 9.2 ± 0.8736 |

Means Given with Standard Errors

| | |
|---|---|
| Average body weight in grams | 19.9 |
| Micrograms D,L-thyroxine secreted per mouse per day | 1.1 |
| Micrograms D,L-thyroxine secreted per 100 grams body weight per day | 5.5 |

TABLE 13 -- THYROID SECRETION RATE, MATURE SCHWING AND ROCKLAND MALE MICE

| Thiouracil in Feed | D,L-Thyroxine Injected per 100 Grams Body Weight per Day | Breed | Number of Animals | Average Body Weight | Average Thyroid Weight | Average Thyroid Weight, Milligrams per 100 Grams Body Weight |
|---|---|---|---|---|---|---|
| per cent | micrograms | | | grams | milligrams | |
| none | none | Schwing | 74 | 20.5 ± 0.4717 | 2.5 ± 0.1243 | 11.6 ± 0.4221 |
| | | Rockland | 10 | 38.0 ± 0.5015 | 5.1 ± 0.1734 | 13.5 ± 0.5138 |
| 0.2 | none | Schwing | 9 | 28.1 ± 2.8519 | 8.9 ± 1.1835 | 30.7 ± 2.5423 |
| | | Rockland | 9 | 36.2 ± 1.2699 | 10.3 ± 0.6288 | 28.7 ± 1.9651 |
| 0.2 | 0.625 | - | - | - | - | - |
| | | Rockland | 5 | 33.2 ± 1.3960 | 6.5 ± 1.4941 | 19.0 ± 3.6069 |
| 0.2 | 1.250 | Schwing | 5 | 30.5 ± 2.5848 | 7.4 ± 1.6121 | 24.5 ± 4.8872 |
| | | Rockland | 5 | 39.0 ± 0.9792 | 7.6 ± 1.1780 | 19.6 ± 1.8500 |
| 0.2 | 2.500 | Schwing | 5 | 28.1 ± 2.0221 | 4.2 ± 0.8131 | 14.7 ± 2.7826 |
| | | Rockland | 5 | 35.6 ± 1.7219 | 6.1 ± 0.8419 | 17.2 ± 1.9563 |
| 0.2 | 3.125 | Schwing | 5 | 25.0 ± 0.5271 | 2.8 ± 0.1143 | 11.0 ± 0.3041 |
| | | Rockland | 5 | 37.1 ± 0.7270 | 4.5 ± 0.1321 | 12.0 ± 0.5007 |

Means Given with Standard Errors

| | Schwing | Rockland |
|---|---|---|
| Average body weight in grams | 22.3 | 36.7 |
| Micrograms D,L-thyroxine secreted per mouse per day | 0.7 | 1.0 |
| Micrograms D,L-thyroxine secreted per 100 grams body weight per day | 3.1 | 2.8 |

TABLE 14 -- THYROID SECRETION RATE, MATURE SCHWING AND ROCKLAND FEMALE MICE

| Thiouracil in Feed | D,L-Thyroxine Injected per 100 Grams Body Weight per Day | Breed | Number of Animals | Average Body Weight | Average Thyroid Weight | Average Thyroid Weight, Milligrams per 100 Grams Body Weight |
|---|---|---|---|---|---|---|
| per cent | micrograms | | | grams | milligrams | |
| none | none | Schwing | 10 | 22.3 ± 1.1044 | 3.4 ± 0.2791 | 15.3 ± 1.1246 |
| | | Rockland | 10 | 33.7 ± 1.6977 | 6.8 ± 1.0323 | 20.1 ± 2.7968 |
| 0.2 | none | Schwing | 5 | 25.3 ± 1.4633 | 10.6 ± 0.6195 | 42.1 ± 2.6979 |
| | | Rockland | 5 | 34.4 ± 2.2972 | 11.0 ± 1.6962 | 32.2 ± 3.7506 |
| 0.2 | 0.625 | - | - | - | - | - |
| | | Rockland | 10 | 31.5 ± 1.0391 | 9.0 ± 0.9703 | 28.2 ± 2.7445 |
| 0.2 | 1.250 | - | - | - | - | - |
| | | Rockland | 10 | 31.4 ± 0.6902 | 8.9 ± 0.9574 | 28.2 ± 2.9659 |
| 0.2 | 2.500 | Schwing | 5 | 24.3 ± 1.6069 | 3.9 ± 0.4757 | 15.8 ± 1.1726 |
| | | Rockland | 5 | 33.5 ± 1.9840 | 5.7 ± 0.5466 | 17.1 ± 1.5244 |
| 0.2 | 5.000 | Schwing | 5 | 24.2 ± 2.4346 | 3.5 ± 0.5075 | 14.4 ± 1.4043 |
| | | Rockland | 5 | 32.8 ± 1.6636 | 4.9 ± 0.5050 | 15.1 ± 1.6857 |
| 0.2 | 10.000 | Schwing | 8 | 25.0 ± 1.0798 | 3.1 ± 0.3154 | 14.1 ± 1.3189 |
| | | - | - | - | - | - |

Means Given with Standard Errors

| | Schwing | Rockland |
|---|---|---|
| Average body weight in grams | 24.0 | 32.7 |
| Micrograms D,L-thyroxine secreted per mouse per day | 0.9 | 0.7 |
| Micrograms D,L-thyroxine secreted per 100 grams body weight per day | 3.6 | 2.1 |

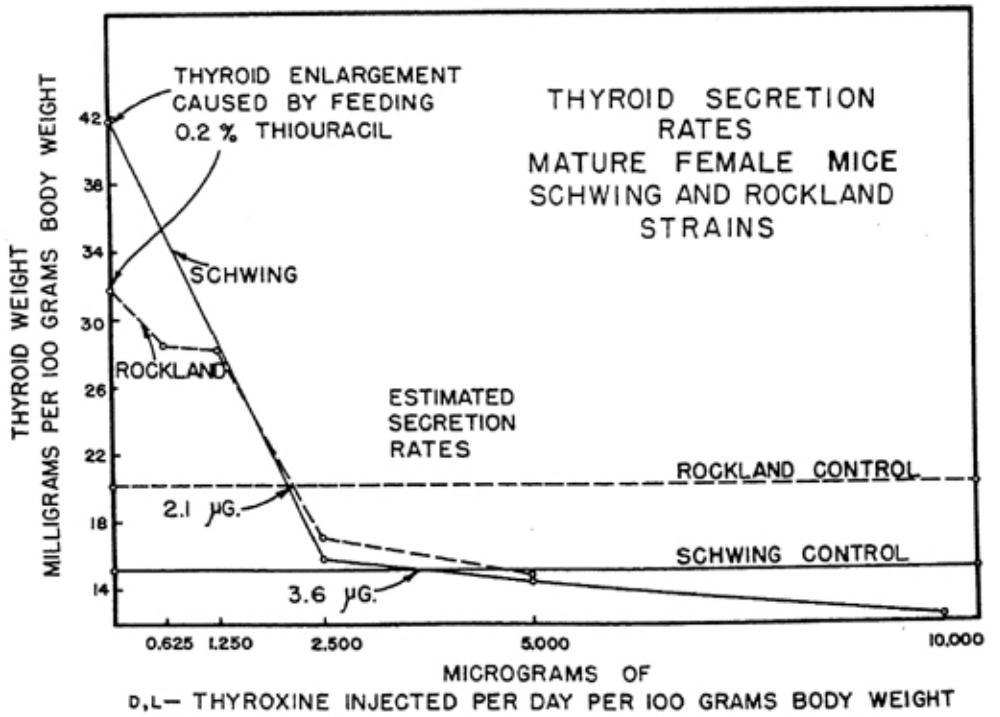


Fig. 9. Thyroid secretion rates, mature female mice, Schwing and Rockland strains.

TABLE 15 -- THYROID SECRETION RATE, MATURE $C_3H$ FEMALE MICE

| Thiouracil in Feed | D,L-Thyroxine Injected per 100 Grams Body Weight per Day | Number of Animals | Average Body Weight | Average Thyroid Weight | Average Thyroid Weight, Milligrams per 100 Grams Body Weight | Mortality |
|---|---|---|---|---|---|---|
| per cent | micrograms | | grams | milligrams | | per cent |
| none | none | 10 | 20.5 ± 0.6645 | 3.3 ± 0.8150 | 15.9 ± 1.0563 | 40 |
| 0.2 | none | 7 | 19.5 ± 0.8090 | 6.2 ± 0.2267 | 31.9 ± 1.5851 | 30 |
| 0.2 | 2.5 | 4 | 17.7 ± 0.8138 | 2.5 ± 0.5131 | 14.0 ± 2.5721 | 20 |
| 0.2 | 5.0 | - | - | - | - | 100 |

Means Given with Standard Errors

| | |
|---|---|
| Average body weight in grams | 19.6 |
| Micrograms D,L-thyroxine secreted per mouse per day | 0.4 |
| Micrograms D,L-thyroxine secreted per 100 grams body weight per day | 2.2 |

## DISCUSSION

The results of these experiments are of interest in that they show that in mature mice there are differences in thyroid secretion rate between strains and also between sexes in a given strain, (Table 16).

### Thyroid Secretion Rates of Different Strains

Mature Schwing female mice secreted the equivalent of 5.5 and 3.6, Rockland females 2.1, and $C_3H$ females 2.2 micrograms of D,L-thyroxine per 100 grams body weight per day. These differences are congruous with the results of Mixner et al., (1944), Schultze and Turner (1945), and Mixner and Upp (1947) in which they observed strain differences in the thyroid secretion rates of different strains of chickens.

The discrepancy between the values obtained for the Schwing female mice may be partially explained in that the animals secreting the larger amount of thyroxine were young mature virgins whereas the animals secreting the smaller amount of thyroxine were mostly parous, older animals. The thyroid secretion rate declines with age, (Monroe and Turner, 1946), and although all of the animals were sexually mature, the thyroid secretion rate appears to decline with age even after growth has stopped. In our colony, mature parous mice attain greater weights than mature nulliparous mice.

Apparently mature body size is no indication of the thyroid secretion rate of a mouse. Mature Rockland female mice, for example, weighed 13 grams more than did mature $C_3H$ female mice and yet their thyroid secretion rates were practically the same. This agrees with Dempsey and Astwood (1943) who observed only minor differences in the thyroid secretion rates of rats of the Long-Evans and Wistar strains. Mature

TABLE 16 -- STRAIN AND SEX DIFFERENCES IN THYROID SECRETION RATES OF MATURE MICE

| Strain | Sex | Number of Animals | Average Body Weight | D,L-Thyroxine Secreted per Mouse per Day | D,L-Thyroxine Secreted per 100 Grams Body Weight per Day |
|---|---|---|---|---|---|
| | | | grams | micrograms | micrograms |
| Schwing | Male | 181 | 22.5 | 0.5 | 2.4 |
| Schwing | Female | 64 | 19.9 | 1.1 | 5.5 |
| Schwing | Male | 98 | 22.3 | 0.7 | 3.1 |
| Schwing | Female | 33 | 24.0 | 0.9 | 3.6 |
| Rockland | Male | 39 | 36.7 | 1.0 | 2.8 |
| Rockland | Female | 45 | 32.7 | 0.7 | 2.1 |
| $C_3H$ | Female | 21 | 19.6 | 0.4 | 2.2 |

Schwing female mice and $C_3H$ mice, weighing approximately the same, had different thyroid secretion rates. Why Rockland mice, weighing eight to 13 grams more than the Schwing mice, produced less thyroxine per 100 grams body weight than did the Schwing mice is a question.

No clear-cut differences are shown between the thyroid secretion rates of mature Schwing and Rockland male mice. The Schwing group secreting the equivalent of 3.1 micrograms of thyroxine per 100 grams body weight per day contained younger animals than did the Schwing group secreting 2.4 micrograms although they were both full-grown and sexually mature. The difference between the thyroid secretion rate of the Rockland mice and either group of Schwing mice, however, is of little significance.

## Differences in Thyroid Secretion Rates Between Sexes

There were differences in thyroid secretion rate between the sexes in both the Schwing and Rockland strains of mice. The curious finding in this work is that whereas in the Schwing strain the females secrete more thyroxine than the males, the situation is reversed in the Rockland strain with the males secreting more thyroxine per 100 grams of body weight than the females.

Schoeller and Gehrke (1927), observed male mice to be more sensitive to thyroxine injections than female mice. We have found the same to be true in both the Schwing and Rockland strains although in the Schwing strain the males produce less thyroxine than the females and in the Rockland strain they produce more.

Rationalization at this point is difficult. The rate of secretion of the thyroid hormone is generally considered to be an index of the animal's tolerance to administered exogenous thyroid hormone. Apparently this does not always hold true.

### Mortality

Mortality is not usually a problem in mice when measuring their thyroid secretion rates unless a large dosage of thyroxine is given. With the $C_3H$ mice used in this study, however, mortality was high in all groups, control and experimental alike.

$C_3H$ mice grow, breed, and lactate well on pellet feed, but apparently when the feed is ground many of the animals die. Autopsies did not yield any pertinent information.

It may be possible that these mice chose the food particles that they wanted and thus did not have a diet with all the essential nutrients. Many animals, however, died on the first day of the experiment, indicating some causative factor which acts more quickly than a dietary deficiency.

## THE RELATION OF THYROID SECRETION RATE TO REPRODUCTIVE FUNCTIONS

### Review of Literature

Hypothyroidism. It has long been recognized that there is a reciprocal relationship between the thyroid gland and the gonads. A decreased secretion of the thyroid hormone is associated with lowered fertility in the ram, (Berliner and Warbritton, 1937, Bogart and Mayer, 1946), in the bovine, (Petersen et al., 1941, Brody and Frankenbach, 1942, Spielman et al., 1945), and in the goat, (Turner et al., 1943). In the fowl, Winchester (1939) observed that thyroidectomy caused a decrease in egg production and Andrews and Schnetzler (1946) reported that feeding thiouracil caused a decrease in testis size in cocks.

Similar observations have been made in laboratory animals. Laqueur and Emge (1941) observed that wheat germ, a goitrogen, caused irregularities in estrus cycles when fed to rats. Goldsmith et al., (1945) prevented rats from becoming pregnant by feeding them thiourea. Mice fed thiourea, (Dalton et al., 1945), or thiouracil, (Morris et al., 1947). show degenerate ovaries. Chu (1945) reported that thyroidectomy interfered with normal pregnancy in the rabbit and Morris et al., (1946) showed that thiourea feeding interfered with estrus cycles in mice. Rogers (1947) found that the feeding of sulfaguanidine reduced litter size in rats. Thyroidectomy, (Engle, 1944), or thiouracil feeding, (Aranow et al., 1946), in monkeys caused prolonged periods of amenorrhea.

Animals that are made hypothyroid experimentally or which are hypothyroid without treatment may be brought to normal sexual function by the administration of thyroid material. Thus the administration of thyroprotein to hypothyroid rams, (Bogart and Mayer, 1946), and to poor breeding bulls, (Reineke, 1946), resulted in marked improvement in fertility. Feeding desiccated thyroid to thyroidectomized bulls restored normal libido, (Peterson et al., 1941).

Hyperthyroidism. The effects of mild hyperthyroidism are generally favorable to sexual functions. Thus, mild hyperthyroidism stimulates egg production in the fowl (Turner, Irwin, and Reineke, 1945, Turner, Kempster, Hall and Reineke, 1945, Turner et al., 1946, and Turner and Kempster, 1947).

The point arises, however, as to what extent one may stimulate an animal above its own thyroid secretion rate and still be within the bounds

TABLE 17 -- EFFECT OF THYROXINE INJECTIONS ON REPRODUCTION IN MICE

| Group | D,L-Thyroxine Injected per 100 Grams Body Weight per Day micrograms | Number of Normal Litters | Number of Still-Born Litters | Mice Not Found Pregnant |
|---|---|---|---|---|
| A 10 Males<br>10 Females | none<br>none | 7 | 0 | 3 |
| B 10 Males<br>10 Females | none<br>200 | 1 | 2 | 7 |
| C 10 Males<br>10 Females | 100<br>none | 7 | 1 | 2 |
| D 7 Males<br>7 Females | 100<br>200 | 1 | 3 | 3 |

of normal physiological response. Van Horn (1933) and Weichert and Boyd (1933) fed large doses of desiccated thyroid to female rats and observed long periods of diestrous. It has been shown in a previous section that as a mouse matures, its thyroid secretion rate declines on the basis of 100 grams body weight. A level of injected thyroxine favorable in stimulating growth at one phase of the growth period is not necessarily favorable to the animal at other times.

## Experimental

Effect of Feeding Thiouracil on Reproduction in Mice. In view of the work already reported, it was of interest to see if thiouracil fed to mice from the time of weaning would prevent or delay the onset of the reproduction period.

Two litters, one with five males and five females, and the other with six males and six females, were placed on 0.2 per cent thiouracil at weaning time. The males and females of each litter were left together in stock cages.

In the cage of five females, one female dropped a litter at the age of 23 weeks and another at 35 weeks. The mice appeared normal at birth but they were eaten within two days by their dams. The young may have died from a lack of nourishment because no milk was observed in them at any time. The other three females in this group did not drop litters.

From the cage of six females, three dropped litters at the ages of 19, 23, and 31 weeks. The litters were not raised but were found partially eaten or drowned in the drinking water. Of the three remaining females, two died and one did not drop a litter.

According to Snell (1941), mice drop their first young at ten to 13 weeks of age. It is apparent from the above results that feeding thiouracil definitely delayed the onset of pregnancy with parturition occurring at 19 weeks of age.

Not all mice on thiouracil fail to drop and raise normal litters. Two female mice receiving thiouracil for three weeks were placed with males and became pregnant within ten days. They dropped and raised litters of normal size and weight.

Reproductive function in male mice did not appear to be affected by feeding thiouracil. This is in agreement with Steiner and Kendall (1946) who observed spermatogenesis to be active in the dog after being treated with thiouracil for one year.

Effect of Hyperthyroidism on Reproduction. Control males and females were taken from stock. The experimental males, beginning at the age of six weeks, had been grown for eight weeks on a ration containing 0.2 per cent thiouracil and injected simultaneously with 100 micrograms of D,L-thyroxine per 100 grams body weight per day. The experimental females, beginning at the age of six weeks, had been grown for eight weeks on a control ration and injected with 200 micrograms of D,L-thyroxine per 100 grams body weight per day.

After the eight week growth period, the males were removed from their thiouracil diet and the males and females were placed together in equal numbers and allowed to breed for four weeks. They were then separated and the females were continued on thyroxine injection and observed for an additional three weeks. At the end of the experiment, all females were continued on thyroxine injection and observed for an additional three weeks. At the end of the experiment, all females which had not dropped litters were then sacrificed and examined for implantation sites or resorbing fetuses. Litters were considered normal if the young appeared healthy and were obtaining milk from their dams.

In this experiment, hyperthyroidism apparently had no effect on the reproductive ability of the male mice, (Table 17).

In female mice, however, hyperthyroidism was markedly detrimental to reproductive function. Hyperthyroid females caged with control males bore one normal litter, two still-born litters, and seven failed to become pregnant. Hyperthyroid females caged with thyroxine-injected males bore one normal litter, three still-born litters, and three failed to become pregnant. Control females, on the other hand, when placed with control males dropped seven normal litters and three failed to breed. Control females placed with hyperthyroid males delivered seven normal litters, one still-born litter, and two failed to breed.

This work shows that the injection of large dosages of thyroxine into the female mouse, although favorable to growth, is detrimental to their breeding ability. This further emphasizes the need for keeping in mind the thyroid secretion rate of an animal when treating it with thyroid materials. Males did not appear to be affected.

## The Effects of Castration on the Thyroid Secretion Rate

Studying the effects of castration on the thyroid secretion rate gives us another means of describing and understanding the thyroid-gonad relationship.

Review of Literature. Although Chouke et al., (1930) in the guinea pig and Zalesky (1935) in the ground squirrel could not find any histological changes in the thyroid gland following castration, Sherwood et al., (1933) have noted a decrease in metabolic rate in the castrated rat, and the same has also been observed in the fowl by Mitchell et al., (1927).

The thyrotrophic content of the anterior pituitary gland was lowered by castration in cattle, (Bates et al., 1935, Reece and Turner, 1937), and in rats, (Turner and Cupps, 1940).

TABLE 18 -- EFFECT OF SHORT-TIME CASTRATION ON THE THYROID SECRETION RATE OF ROCKLAND MALE MICE

| Thiouracil in Feed | D,L-Thyroxine Injected per 100 Grams Body Weight per Day | Group | Number of Animals | Average Body Weight | Average Thyroid Weight | Average Thyroid Weight, Milligrams per 100 Grams Body Weight |
|---|---|---|---|---|---|---|
| per cent | micrograms | | | grams | milligrams | |
| none | none | Control | 8 | 19.1 ± 1.1250 | 1.9 ± 0.2358 | 10.3 ± 0.9335 |
| | | Castrate | 8 | 20.3 ± 1.1603 | 2.8 ± 0.3925 | 13.8 ± 1.3700 |
| 0.2 | none | Control | 8 | 20.8 ± 1.1601 | 9.4 ± 0.7666 | 45.1 ± 2.7042 |
| | | Castrate | 7 | 21.2 ± 0.6331 | 6.1 ± 0.5621 | 29.0 ± 2.5577 |
| 0.2 | 0.25 | Control | 8 | 20.7 ± 0.7046 | 6.6 ± 0.5334 | 31.7 ± 2.7892 |
| | | Castrate | 7 | 20.9 ± 0.7267 | 8.1 ± 0.6457 | 38.8 ± 2.8741 |
| 0.2 | 0.50 | Control | 5 | 21.2 ± 1.3759 | 4.1 ± 0.8302 | 19.3 ± 2.7454 |
| | | Castrate | 7 | 21.5 ± 1.5497 | 4.8 ± 1.2681 | 22.3 ± 4.5629 |
| 0.2 | 1.00 | Control | 8 | 22.8 ± 1.2097 | 5.9 ± 0.8064 | 25.7 ± 3.2480 |
| | | Castrate | 6 | 23.3 ± 0.8141 | 5.5 ± 0.8309 | 23.6 ± 2.7089 |
| 0.2 | 2.00 | Control | 8 | 21.9 ± 0.6528 | 3.4 ± 0.4894 | 15.5 ± 2.0920 |
| | | Castrate | 5 | 21.1 ± 1.9503 | 2.9 ± 0.5208 | 13.9 ± 2.3195 |

Means Given with Standard Errors

| | Control | Castrate |
|---|---|---|
| Average body weight in grams | 21.1 | 21.3 |
| Micrograms D,L-thyroxine secreted per mouse per day | 2.5 | 2.0 |
| Micrograms D,L-thyroxine secreted per 100 grams body weight per day | 0.5 | 0.4 |

Schultze and Turner (1945) observed that castration lowered the thyroid secretion rate in chickens which had been caponized eight weeks prior to the beginning of the experiment.

Experimental. Two series of assays were run to determine the effect of castration on the thyroid secretion rate in mice. The first, (Table 18), was carried out on mature male Schwing mice. The animals were castrated seven days prior to the beginning of the experiment. The normal males secreted the equivalent of 2.5 micrograms of D,L-thyroxine per 100 grams body weight per day and the castrated males secreted 2.0 micrograms. The results showed little if any difference between the two groups.

It was thought that the work should be repeated using a longer period between the time of castration and the assay period. Turner and Cupps (1940) showed that the thyrotrophic content of the pituitary of the rat declined following castration as a function of time.

In the second experiment, therefore, 16 weeks were allowed to pass following castration before the mice were placed on experiment.

It was indicated in the second trial that castration reduces the thyroid secretion rate.

## The Effects of Feeding Dianisylhexene (Dimethyl Ether of Diethylstilbestrol) on the Thyroid Secretion Rate

If the withdrawal of sex hormones results in a lowered rate of thyroid hormone secretion, it would be reasonable to expect that an increased supply of sex hormones would increase the thyroid secretion rate.

Review of Literature. Leiby (1933) observed thyroid hypertrophy in spayed female rats injected with estriol and Anderson (1934) noted that the atrophic changes in the thyroid of the spayed female rat may be restored to normal with a physiological dose of estrogen.

Although Emmens (1938) showed that the fowl thyroid is depressed following massive dosages of estrogen, Knude et al., (1930) had reported estrogen administration as having a stimulating thyroid effect in the dog.

Schultze and Turner (1945) showed that the feeding of dianisylhexene for five weeks prior to the assay period increased the thyroid secretion rate in Barred Rock pullets.

Experimental. Rockland female mice, starting at approximately six weeks of age, were fed a diet containing 0.3 milligrams of dianisylhexene per kilogram of feed for 13 weeks prior to the assay period.

The level of dianisylhexene fed was determined from the work of Trentin and Turner (1948). They have shown that this level of dianisylhexene is effective in that it stimulates the mammary growth of growing female mice and yet is not enough to interfere with the normal growth process.

Normal females secreted the equivalent of 2.1 micrograms of D,L-thyroxine per 100 grams of body weight and dianisylhexene-treated females secreted 3.6 micrograms. Dianisylhexene feeding in physiological dosages increased the thyroid secretion rate of female mice, (Table 19, Figure 10).

## DISCUSSION

Work in this section has indicated the possibility that castration depresses and mild estrogen administration stimulates the thyroid secretion rate in mice. Since the only known direct metabolic activator of thyroid hormone formation is the thyrotrophic hormone which is secreted by the anterior pituitary gland, it follows that the action of the sex hormones on thyroid secretion rate is mediated by way of their influence on pituitary thyrotrophin secretion.

Since the thyroid-gonad relationship is reciprocal, it is of interest to speculate on the manner in which thyroxine influences gonad function.

Leatham (1945) found that feeding thiourea to female rats increased the gonadotrophic content of their pituitaries but later Leatham (1947) observed that thiouracil feeding did not affect the gonadotrophic content of rat pituitaries.

Stein and Lisle (1942), however, found a decrease in the gonad stimulating potency of young male rats following thyroidectomy. Chu and You (1945) gave thyroid to rabbits and found that the pituitary follicle stimulating hormone was lowered and the lutinizing hormone increased. Further work is needed to clarify the manner by which the thyroid gland affects the gonads.

## THE THYROID SECRETION RATE AND LACTATION

Lactation represents a period during which the mammal must not only maintain itself but it must also secrete milk in sufficient quantities to rear its young.

Following the initiation of lactation, associated with a marked increase in the secretion of the lactogenic hormone by the pituitary gland,

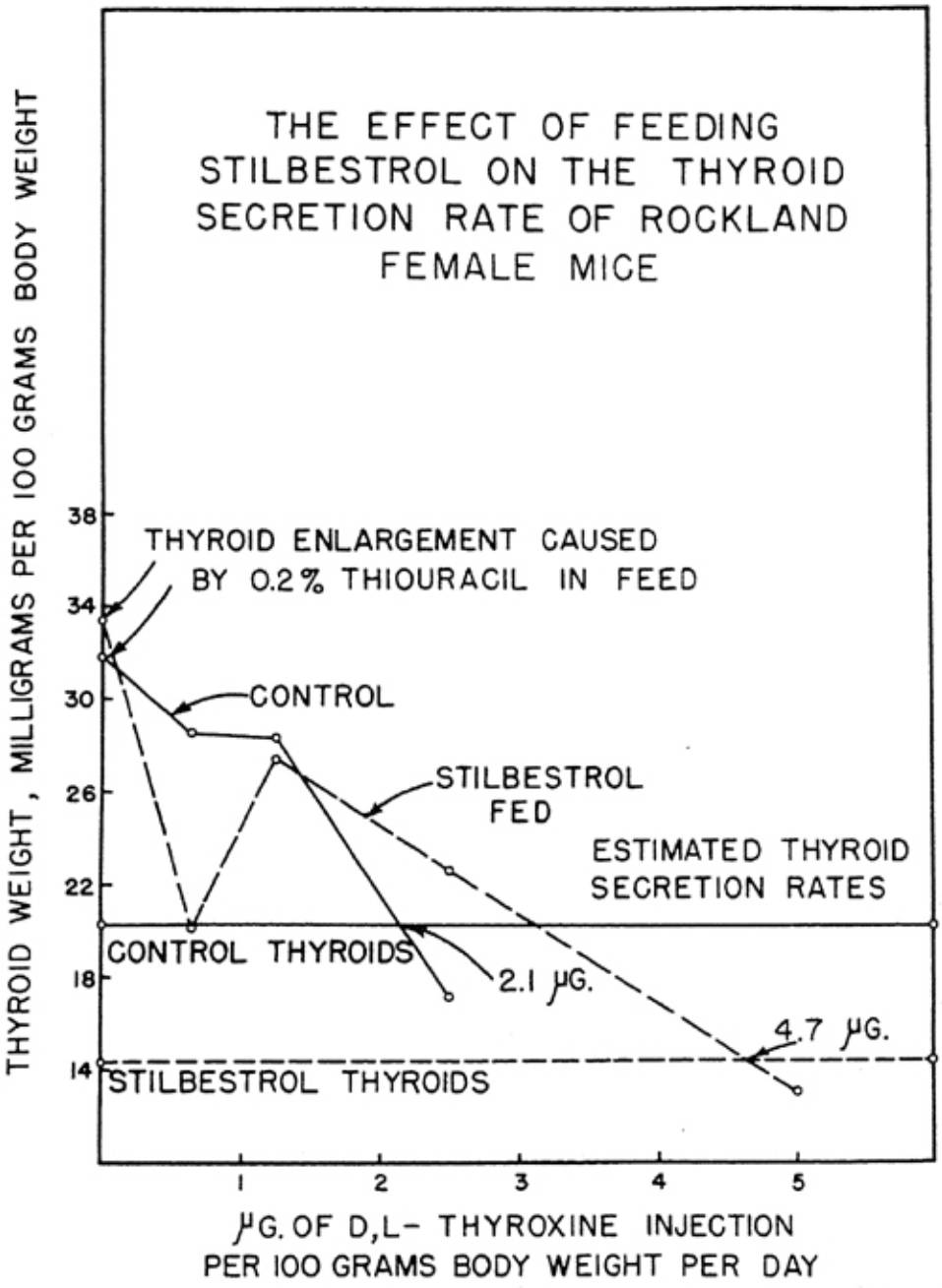


Fig. 10. Stilbestrol refers to dimethyl ether of diethylstilbestrol.

(Meites and Turner, 1942a, 1942b, 1942c), the maintenance of lactation is due in part to the increased activity of the animal's endocrine glands.

The pituitary secretes the lactogenic hormone in larger amounts during lactation than during any other period, (Hurst and Turner, 1942). Lactogenic hormone production coincides fairly well with the lactation curve. The question has been raised as to whether or not the thyroid gland exercises any control over the secretion of the lactogenic hormone by the anterior pituitary gland. Injecting thyroxine alone in the rat, (Reece and Turner, 1937) or in the mouse, (Hurst and Turner, 1942) did not affect pituitary lactogen content. The theory that thyroxine might augment the effect of estrogen in increasing pituitary lactogen was not borne out in the rat by Meites and Turner (unpublished data). These workers, however, used a very small dosage of thyroxine, and this may account for their results.

TABLE 19 -- EFFECT OF FEEDING DIANISYLHEXENE (DIMETHYL ETHER OF DIETHYLSTILBESTROL) ON THYROID SECRETION RATE OF FEMALE ROCKLAND MICE

| Thiouracil in Feed | D,L-Thyroxine Injected per 100 Grams Body Weight per Day | Group | Number of Animals | Average Body Weight | Average Thyroid Weight | Average Thyroid Weight, Milligrams per 100 Grams Body Weight |
|---|---|---|---|---|---|---|
| per cent | micrograms | | | grams | milligrams | |
| none | none | Control | 10 | 33.7 ± 1.6977 | 6.8 ± 1.0323 | 20.1 ± 2.7968 |
| | | Stilbestrol | 11 | 32.6 ± 0.8120 | 4.7 ± 0.3908 | 14.3 ± 1.0044 |
| 0.2 | none | Control | 5 | 34.4 ± 2.2972 | 11.0 ± 1.6962 | 32.2 ± 3.7506 |
| | | Stilbestrol | 10 | 34.3 ± 1.0255 | 11.5 ± 0.6880 | 33.4 ± 1.3176 |
| none | 1.250 | Stilbestrol | 9 | 33.1 ± 0.8972 | 5.3 ± 0.1888 | 16.1 ± 0.4644 |
| 0.2 | 0.625 | Control | 10 | 31.5 ± 1.0391 | 9.0 ± 0.9703 | 28.2 ± 2.7445 |
| | | Stilbestrol | 9 | 32.0 ± 1.0423 | 6.6 ± 1.1276 | 20.1 ± 2.8438 |
| 0.2 | 1.250 | Control | 10 | 31.4 ± 0.6902 | 8.9 ± 0.9574 | 28.2 ± 2.9659 |
| | | Stilbestrol | 10 | 32.7 ± 1.5007 | 8.9 ± 1.0676 | 27.3 ± 3.1959 |
| 0.2 | 2.500 | Control | 5 | 33.5 ± 1.9840 | 5.7 ± 0.5466 | 17.1 ± 1.5244 |
| | | Stilbestrol | 11 | 30.2 ± 1.1165 | 6.9 ± 1.0007 | 22.5 ± 3.1084 |
| 0.2 | 5.000 | Control | 5 | 32.8 ± 1.6636 | 4.9 ± 0.5050 | 15.1 ± 1.6857 |
| | | Stilbestrol | 10 | 34.6 ± 1.8412 | 4.5 ± 0.5010 | 13.1 ± 1.3030 |

Means Given with Standard Errors

| | Control | Dianisylhexene |
|---|---|---|
| Average body weight in grams | 32.7 | 32.7 |
| Micrograms D,L-thyroxine secreted per mouse per day | 0.7 | 1.5 |
| Micrograms D,L-thyroxine secreted per 100 grams body weight per day | 2.1 | 4.7 |

On the other hand, there is evidence to show that hypothyroidism decreases the pituitary lactogen content. Meites and Turner (1947) have observed that although the simultaneous administration of thiouracil and estrogen to rats did not lower pituitary lactogen content over a short-time period, the pre-treatment of the animals with thiouracil followed by the simultaneous administration of thiouracil plus estrogen did decrease pituitary lactogen. Thiouracil administration causes a reduction in the acidophil cells of the pituitary which secrete the lactogenic hormone. Thus, estrogen, which is a powerful augmenter of pituitary lactogen, has few cells on which it can then exert its effect.

## The Role of the Thyroid Gland in Lactation

Laboratory Animals. Most of the work reported on the relationship of the thyroid gland to lactation in laboratory animals has been carried out on the rat. Evidence previously cited indicates that the rat, as contrasted to the mouse, grows more rapidly and undergoes greater mammary development when in a slightly hypothyroid condition. There is no direct evidence at this point to show that a slight degree of hypothyroidism will stimulate lactation in the rat.

All work reported to date on the effect of lowered energy metabolism on lactation in the rat has been accompanied by thyroidectomy which would reduce the amount of available thyroxine to almost nil. Thus, although Nelson and Tobin (1937) and Nelson (1939) reported that thyroidectomy did not interfere with lactation in the rat, Folley (1938), Preheim (1940), Folley et al., (1942), and Karnofsky (1942) reported that thyroidectomy did depress lactation in the rat.

TABLE 20 -- THE EFFECT OF THE SIMULTANEOUS ADMINISTRATION OF THYROXINE AND THIOURACIL ON LACTATING MICE

| Thiouracil in Feed | D,L-Thyroxine Injected per 100 Grams Body Weight per Day | Number of Animals | Average Body Weight | Average Thyroid Weight | Average Thyroid Weight, Milligrams per 100 Grams Body Weight |
|---|---|---|---|---|---|
| per cent | micrograms | | grams | milligrams | |
| none | none | 10 | 34.5 ± 1.1025 | 4.4 ± 0.3663 | 12.8 ± 1.2230 |
| 0.2 | none | 4 | 37.6 ± 2.0201 | 10.8 ± 2.5946 | 28.4 ± 5.8316 |
| 0.2 | 3.0 | 10 | 36.3 ± 0.8569 | 3.5 ± 0.3349 | 9.7 ± 1.0999 |
| 0.2 | 4.0 | 6 | 36.3 ± 0.8818 | 3.8 ± 0.4695 | 10.3 ± 1.1817 |
| 0.2 | 6.0 | 5 | 35.7 ± 1.5673 | 4.3 ± 0.6192 | 11.9 ± 1.1964 |

Farm Animals. Hypothyroidism. Graham (1934) in the cow and Ralston et al., (1940) in the goat have shown that thyroidectomy depresses lactation. Schultze and Turner (1945) observed that feeding thiourea or thiouracil to goats or thiourea to cows depressed lactation. Milk production was restored to normal in the goats and cows when given thyroxine.

Mild hyperthyroidism. That thyroxine injections cause an increase in milk production in the dairy cow has been shown by Graham (1934), Jack and Bechdel (1935), Jones (1935), Folley and White (1936), Folley and Young (1938), Herman et al., (1938), Ralston et al., (1940), Hurst (1940), and Smith and Dastur (1940). Thyroxine injections also stimulate milk production in the dairy goat, (Ralston et al., 1940).

Thyroprotein feeding to the dairy cow has also increased milk production, shown by Reineke and Turner (1942), Blaxter (1943), Van Landingham et al., (1944), Reece (1944), Seath et al., (1945), Archibald (1945), Blaxter (1945), Blaxter (1946), Kemmerer et al., (1946), Reece (1947), Hibbs and Krauss (1947), and Booth et al., (1947).

## Experimental

The thyroid secretion rate was studied in Rockland mice at the twenty-first day of lactation. Young were kept with their dams during the assay period.

It was observed that the thyroid glands of the animals fed thiouracil and injected simultaneously with thyroxine all weighed less than the control thyroids, (Table 20). Thyroid secretion in lactating female mice at the twenty-first day of lactation is somewhat less than the equivalent of three micrograms of D,L-thyroxine per 100 grams body weight per day. This would indicate that thyroid secretion rate is not increased over control values at the twenty-first day of lactation.

## Discussion

These results agree with those of Schultze and Turner (1945) in that they found no significant differences in thyroid secretion rate between

dry and lactating goats although they did show that thyroid secretion rate varied directly according to production in lactating goats.

Monroe and Turner (1946) reported that in the rat there is no difference in thyroid secretion rate between non-lactating rats and those in the sixteenth day of lactation.

The twenty-first day of lactation in the mouse may not represent the peak of the thyroid secretion rate during lactation inasmuch as Enzmann (1933) has shown that in the mouse the peak of the lactation curve is reached at the eleventh and twelfth days post-partum. The concentration of the lactogenic hormone in the pituitary has declined by the twenty-first day of lactation in the mouse, (Hurst and Turner, 1942).

Although the thyroid gland may be a limiting factor in lactation, the thyroid secretion rate of the mouse did not show any increase over non-lactating mice at the twenty-first day post-partum.

## The Non-Permeability of the Mammary Gland to Thyroxine

The possibility of the passage of thyroxine into the milk of mice was studied in two experiments.

In the first experiment, lactating female mice were fed 0.2 per cent thiouracil in the feed and injected simultaneously with ten micrograms of D,L-thyroxine per 100 grams body weight per day beginning at the first day post-partum. The mothers with their young were sacrificed on the twelfth day post-partum. The thyroids of the young were hyperemic and enlarged whereas the thyroids of the dams appeared to be atrophic.

In the second experiment, lactating female mice were divided as follows: one control group, one group received 0.2 per cent thiouracil in the feed, and the three remaining groups received 0.2 per cent thiouracil in the feed and were simultaneously injected with thyroxine. One group received three, one four, and the other six micrograms of D,L-thyroxine per 100 grams body weight per day. In all groups the young were allowed to suckle until day 21 post-partum.

Although the thyroid glands of all the thyroxine-injected dams were smaller than those of the controls, the thyroid glands of their litters had enlarged thyroids, (Table 21).

## Discussion

The material presented in this section tends to confirm the work of Monroe and Turner (1946) and others in that no evidence can be found that thyroxine is passed through the mammary gland into the milk. Thiouracil passes readily into the milk. Williams et al., (1944) have found higher concentrations of thiouracil in the milk than in any other body tissue or fluid.

It may be argued that the young sacrificed at day 21 post-partum may have received thyroxine in the milk but that it was not enough to counteract the large amounts of thiouracil which they obtained by eating grain feed. Young Rockland mice begin to eat grain feed as early as 12 days post-partum and can be weaned at 15 days post-partum. The dams all received thyroxine in excess of their own thyroid secretion rates, and it would appear that thyroxine in these concentrations would have been able to counteract any effects of thiouracil in the young. The young, although secreting more thyroxine than older mice on the basis of 100

TABLE 21 -- THE NON-PERMEABILITY OF THE MAMMARY GLAND TO THYROXINE

| Treatment of Dams | | | | | | | |
|---|---|---|---|---|---|---|---|
| Thiouracil in Feed | D,L-Thyroxine Injected per 100 Grams Body Weight per Day | Number of Litters | Average Number Weaned per Litter | Average Body Weight of Young | Average Thyroid Weight of Young | Average Thyroid Weight of Young, Milligrams per 100 Grams Body Weight | Average Thyroid Weight of Dams, Milligrams per 100 Grams Body Weight |
| per cent | micrograms | | | grams | milligrams | | |
| none | none | 10 | 4.5 | 10.8 ± 0.4051 | 1.9 ± 0.1070 | 17.1 ± 0.7923 | 12.8 ± 1.2230 |
| 0.2 | none | 4 | 4.5 | 10.6 ± 0.3735 | 10.9 ± 0.4269 | 103.8 ± 4.4837 | 28.4 ± 5.8316 |
| 0.2 | 3.0 | 10 | 5.5 | 10.0 ± 0.2920 | 7.0 ± 0.3164 | 69.7 ± 0.8288 | 9.7 ± 1.0999 |
| 0.2 | 4.0 | 6 | 4.7 | 9.8 ± 0.3847 | 6.8 ± 0.7076 | 68.1 ± 5.4288 | 10.3 ± 1.1817 |
| 0.2 | 6.0 | 5 | 4.6 | 10.2 ± 0.2949 | 10.7 ± 0.4212 | 105.6 ± 3.5082 | 11.9 ± 1.1964 |

Means Given with Standard Errors

grams body weight, would still secrete only small amounts when their body weights are taken into consideration and thus require only small amounts to overcome the effects of thiouracil.

Variations may be noted in the thyroid weights of the young. There is always considerable variation in the response of mouse thyroids to thiouracil and the variation is always greater in young growing animals than it is in mature animals. It can be observed that litters of mice receiving the highest dosage of thyroxine had the largest thyroids, (Table 21).

When the young were sacrificed at day 12 post-partum before they had an opportunity to eat grain feed, their thyroids were obviously enlarged although those of the dams were atrophic when receiving thiouracil and thyroxine simultaneously.

It is believed that this evidence supports the contention that thyroxine does not pass through the mammary gland into the milk.

## THE RELATION OF ENVIRONMENTAL TEMPERATURE TO THYROID SECRETION RATE

At 80° Fahrenheit, mature Schwing male mice averaging 22.5 grams in body weight secreted the equivalent of 0.54 micrograms of D,-L-thyroxine per day and 2.4 micrograms per 100 grams body weight whereas at 87° Fahrenheit mature Schwing male mice averaging 23.6 grams in body weight secreted the equivalent of 0.17 micrograms of D,-L-thyroxine per day and 0.7 micrograms per 100 grams body weight, (Tables 22 and 23).

At 80° Fahrenheit, mature Schwing female mice averaging 19.9 grams in body weight secreted the equivalent of 1.09 micrograms of D,L-thyroxine per day and 5.5 micrograms per 100 grams body weight whereas at 87° Fahrenheit mature Schwing female mice averaging 21.4 grams in body weight secreted the equivalent of 0.68 micrograms of D,L-thyroxine per day and 3.2 micrograms per 100 grams body weight, (Tables 22 and 23).

TABLE 22 -- THYROID SECRETION RATE AT 87 DEGREES FAHRENHEIT, MATURE SCHWING MICE

| Thiouracil in Feed per cent | D,L-Thyroxine Injected per 100 Grams Body Weight per Day micrograms | Number of Animals | Average Body Weight grams | Average Thyroid Weight milligrams | Average Thyroid Weight, Milligrams per 100 Grams Body Weight |
|---|---|---|---|---|---|
| | | | Males | | |
| none | none | 9 | 26.1 ± 1.3040 | 3.8 ± 0.4434 | 14.7 ± 1.4938 |
| 0.2 | none | 7 | 24.2 ± 0.6631 | 7.3 ± 1.0247 | 30.3 ± 3.7811 |
| 0.2 | 0.5 | 10 | 21.6 ± 0.4052 | 3.5 ± 0.3703 | 16.3 ± 1.6724 |
| 0.2 | 1.0 | 11 | 23.1 ± 0.9612 | 2.8 ± 0.2414 | 12.0 ± 1.1217 |
| | | | Females | | |
| none | none | 9 | 20.6 ± 0.5646 | 2.8 ± 0.1847 | 13.6 ± 0.7827 |
| 0.2 | none | 10 | 21.5 ± 0.5224 | 8.5 ± 1.1543 | 39.7 ± 5.7914 |
| 0.2 | 1.0 | 9 | 21.5 ± 1.0000 | 5.0 ± 0.6217 | 23.2 ± 2.6646 |
| 0.2 | 2.0 | 10 | 22.1 ± 0.2739 | 3.6 ± 0.3444 | 16.1 ± 1.4620 |
| 0.2 | 4.0 | 9 | 21.2 ± 0.6218 | 2.5 ± 0.2117 | 11.9 ± 0.8315 |

Means Given with Standard Errors

| | Males | Females |
|---|---|---|
| Average body weight in grams | 23.6 | 21.4 |
| Micrograms D,L-thyroxine secreted per mouse per day | 0.17 | 0.68 |
| Micrograms D,L-thyroxine secreted per 100 grams body weight per day | 0.70 | 3.20 |

TABLE 23 -- THE RELATION OF ENVIRONMENTAL TEMPERATURE TO THYROID SECRETION RATE

| Body Weight grams | Temperature, Degrees Fahrenheit | Number of Animals | D,L-Thyroxine Secreted per Mouse per Day micrograms | D,L-Thyroxine Secreted per 100 Grams Body Weight per Day micrograms |
|---|---|---|---|---|
| | | Males | | |
| 22.5 | 80 | 181 | 0.54 | 2.40 |
| 23.6 | 87 | 37 | 0.17 | 0.70 |
| | | Females | | |
| 19.9 | 80 | 64 | 1.09 | 5.50 |
| 21.4 | 87 | 47 | 0.68 | 3.20 |

TABLE 24 -- AMOUNT OF THYROPROTEIN REQUIRED TO MAINTAIN NORMAL THYROID SIZE IN MATURE MICE FED 0.2 PER CENT THIOURACIL

| Strain | Sex | Number of Animals | Average Body Weight | Average Daily Food Consumption per Mouse | Thyroprotein Required in Feed |
|---|---|---|---|---|---|
| | | | grams | grams | per cent |
| Schwing | Female | 48 | 21.1 | 3.1 | 0.01 |
| Rockland | Female | 45 | 34.8 | - | 0.01 |

## Discussion

Temperature changes affect the thyroid secretion rate in the mouse. This agrees with work reported on the rat by Dempsey and Astwood (1943).

Herrington (1941) studied the basal metabolism in the mouse and found the "zone of thermoneutrality," defined by Brody (1945) as "the temperature at which heat loss from the body is equal to the minimum heat production," to be between 85° and 92° Fahrenheit. On this basis, the mice kept at 80° Fahrenheit would increase their basal energy metabolism in order to compensate for heat losses from the body. This was found to be true, since in both sexes the thyroid secretion rate was increased at 80° Fahrenheit as compared to 87°.

## THE UTILIZATION OF THYROPROTEIN BY THE MOUSE

In experimental work it may become desirable under certain conditions to administer thyroxine orally to mice rather than to administer it by injection. Thus, in long-time growth experiments or in experiments where large numbers of animals are involved, the work would be simplified by being able to incorporate the thyroid hormone into the feed.

Thyroprotein, a synthetic iodinated-casein containing 2.91 per cent L-thyroxine, is well suited for this work.

Extensive studies have been carried out in determining the thyroid secretion rate in various animals by noting the amount of injected thyroxine required to maintain normal-sized thyroid glands when the animal is fed simultaneously with thiouracil.

If thyroprotein mixed in the feed were substituted for injected thyroxine in this assay, the utilization of the thyroxine in the thyroprotein by the animal could be estimated.

## Experimental

Mature Schwing female mice averaging 21.2 grams in body weight and mature Rockland female mice averaging 34.8 grams require 0.01 per cent thyroprotein in the feed to maintain normal-sized thyroid glands when fed simultaneously with thiouracil, (Table 24).

It was observed that mature Schwing female mice on control feed and on feed containing 0.01 per cent thyroprotein and 0.2 per cent thiouracil consumed 3.1 grams of feed daily per animal.

Mice consuming 3.1 grams of feed containing 0.01 per cent thyroprotein would consume 310 micrograms of thyroprotein per day. In 310 micrograms of thyroprotein there would be nine micrograms of L-thyroxine or the equivalent of 18 micrograms of D,L-thyroxine. Since the mice averaged 21.1 grams in body weight, they would be consuming the equivalent of 85 micrograms of D,L-thyroxine per 100 grams body weight per day.

With the mature virgin Schwing female mouse secreting the equivalent of 5.5 micrograms of D,L-thyroxine per 100 grams body weight per day, it would follow that approximately seven per cent of the thyroxine in thyroprotein is utilized when it is administered orally to the mouse as compared to subcutaneous injection. This agrees closely with Turner and Reineke (1946) who showed that sheep utilized approximately five per cent of the thyroxine in thyroprotein when given orally as compared to subcutaneous injection.

### Discussion

Schultze and Turner (1945) found that the chicken requires 0.009 per cent thyroprotein in the feed to maintain normal thyroid size when thiouracil is fed simultaneously. This agrees closely with the mouse which requires 0.01 per cent.

It is believed that these figures may serve as a guide in feeding thyroprotein to laboratory animals. It is important here, as it is in the case where thyroxine is injected, that the thyroid secretion rate be taken into account in order to remain within physiological dosage levels.

## DISCUSSION

Work presented in this paper supports the concept that the knowledge of the thyroid secretion rate in an animal aids in understanding the relationship of the thyroid gland to various physiological functions. This is important since the thyroid gland plays an important role in the physiological processes of growth, reproduction, and lactation, and it is also a factor in adjusting the animal to changes in environmental temperatures, in aiding in the absorption of sugar from the intestine, and in general acting as a metabolic regulator of all body processes.

The importance of the thyroid gland during growth is well known. Its function during growth is better understood, however, if it can be quantitatively expressed in terms of the amount of thyroid hormone that is being secreted. The terms hypo- and hyperthyroidism are broad and rather meaningless. The measures of function of other glands or organs such as the heart, kidney, or lungs are never mentioned in such broad aspects as "hypo" or "hyper," and the measure of thyroid function should not be an exception. During growth, for example, it has been observed that the thyroid secretion rate in the mouse declines as the animal reaches maturity.

These observations are of interest since the mouse has been used extensively for the study of thyroid activity. In these laboratories we have observed that hyperthyroidism can be both detrimental and beneficial to the growing mouse. Such a statement appears contradictory coming from one laboratory, but such a statement could be made of the general literature and the reader would be left in the position of not appreciating the function of the thyroid during growth. This statement can be qualified, however, by stating that stimulating the growing mouse by 80

times its own thyroid secretion rate is detrimental to growth, but stimulating the growing mouse by 20 to 60 times its own thyroid secretion rate is beneficial to growth. Quantitative expressions lend clarity to a report of physiological activity, and this concept would lead to clearer thinking in regard to thyroid function during growth, not only in the mouse, but in other species of laboratory and farm animals as well.

In addition to the decline in thyroid secretion rate during the growth period, there are also indications to show that best results for stimulating growth are gained by reducing the amount of thyroid hormone administered in accordance with declining thyroid secretion rate. More work needs to be done, however, to support this contention.

The thyroid gland is closely linked to the function of the gonads. Here again, there have been numerous statements regarding the activity of the gonads during hypo- or hyperthyroidism, and also regarding thyroid activity during various stages of gonad function. The work presented here on the mouse confirms the work of Schultze and Turner (1945) in the chicken in that castration lowers and estrogen administration increases the thyroid secretion rate. This illustrates a point of value in determining the thyroid secretion rate not only for a quantitative estimate, but also to give a clearer concept as to just why, for example, castration may retard growth. On the basis of these studies it can be said that growth retardation following castration is due in part, at least, to a lowered thyroid secretion rate.

Another point of interest is the relation of the change in thyroid secretion rate in response to changes in environmental temperature. This was first observed by Dempsey and Astwood (1943) in the rat.

Many animals, when exposed to a temperature outside of the zone of thermoneutrality, must alter their basal energy metabolism. In temperatures below the zone of thermoneutrality their metabolic processes must increase in order to maintain body temperature, and above this zone their metabolic processes increase in an attempt to dissipate additional amounts of heat. These changes are made, it appears, by the variation in the animal's rate of thyroid secretion and in mice, thyroid secretion rate was increased as the environmental temperature was lowered below that of their range of thermoneutrality.

All of these factors considered point to one concept; namely, that in order to consider the relation of thyroid function to various physiological processes, the thyroid secretion rate must be taken into account.

## SUMMARY

1. Thyroid secretion rate was measured in mice by a technique in which D,L-thyroxine is injected daily into the animal and thiouracil is administered orally as a part of the ration. The thiouracil administered prevents the synthesis of thyroxine by the thyroid gland and the resultant lowered thyroxine level in the blood stimulates the secretion of thyrotrophin by the pituitary gland. This increased thyrotrophin secretion in turn causes thyroid enlargement. Replacing the level of thyroxine normally found in the blood by daily thyroxine injection maintains pituitary thyrotrophin secretion at a normal level. The result, then, of the concurrent feeding of thiouracil and injection of thyroxine in dosages equal to the normal thyroid secretion rate is to maintain a

thyroid gland comparable in size to thyroid glands of control animals. This measure was then used to study the relationship of the thyroid secretion rate to various physiological processes such as growth, reproduction, lactation, and change in environmental temperature.

2. It was found that the incorporation of 0.05 or 0.1 per cent thiouracil into the feed for a two week period did not significantly increase the weight of the thyroid gland of the mature mouse. The addition of 0.2 per cent thiouracil to the feed, however, did increase thyroid size significantly during a two week period.

3. Feeding 0.05, 0.1, or 0.2 per cent thiouracil to young mice for a two week period immediately following weaning retarded growth. Feeding 0.2, 0.4, and one per cent thiouracil to young mice for a six week period immediately following weaning retarded growth. Feeding 0.1 per cent thiouracil to young male mice for ten weeks and to young female mice for 12 weeks appeared to retard growth although by the end of the feeding trials there were no significant differences between control and experimental body weights. Carcass analyses were made on the female mice and it was found that the thiouracil-fed mice contained 3.4 per cent more fat than did the control mice. Since true growth may be represented by nitrogenous gain, it appears that the rate of true growth may have been retarded. There were no significant differences in right tibia lengths between the control and experimental groups.

4. Growing female mice received 0.1 per cent thiouracil in the feed and were injected daily with D,L-thyroxine in amounts calculated on the basis of dosage per 100 grams body weight. Those receiving four and eight micrograms of thyroxine, which approximated normal thyroid secretion rate, maintained normal growth gains. Injecting 100 micrograms of thyroxine stimulated growth above controls by the end of the fourth week of experiment. Injecting 200 micrograms did not appear to affect growth but at the end of ten weeks the experimental animals were significantly smaller than the controls. Injecting 300 micrograms stimulated growth above normal after the animals had been on experiment two weeks. Injecting 400 micrograms retarded growth. Growing male mice were not affected by the injection of eight micrograms of thyroxine in a similar experiment, but injecting 100 micrograms retarded growth.

5. There were some indications that the reduction of thyroxine dosage during a rapid period of growth coinciding with a declining thyroid secretion rate stimulated growth to a greater extent than did the administration of a constant dosage of thyroxine over the same eight week period.

6. The thyroid secretion rate of growing yellow female mice did not differ significantly from that of growing albino female mice which had similar growth curves.

7. The thyroid secretion rate per 100 grams body weight declined during the growth period.

8. The thyroid secretion rates in terms of D,L-thyroxine equivalent per 100 grams body weight per day for mature virgin female mice were as follows: $C_3H$ strain averaging 19.6 grams in body weight, 2.2 micrograms; Schwing strain averaging 19.9 grams in body weight, 5.5 micrograms; and Rockland strain averaging 32.7 grams in body weight, 2.1 micrograms. The results for mature male mice were as follows: Schwing strain averaging 22.4 grams in body weight, 2.4 micrograms;

Rockland strain averaging 36.7 grams in body weight, 2.8 micrograms. The Rockland mice are not only larger mice than those in the $C_3H$ and Schwing strains, but they also grow more rapidly.

9. Feeding thiouracil to female mice adversely affected gonad function. Injecting thyroxine in amounts above physiological dosage levels as compared to the thyroid secretion rate also adversely affected gonad function.

10. Thyroid secretion rate in male mice was not lowered in castrated male mice if only seven days were allowed to elapse between the time of castration and the measurement of thyroid secretion rate, but thyroid secretion rate was lowered in castrated male mice if 16 weeks were allowed to elapse between the time of castration and the measurement of thyroid secretion rate.

11. The feeding of dianisylhexene at the rate of 0.3 milligrams per kilogram of feed for 13 weeks prior to assay, increased the thyroid secretion rate of mature Rockland female mice to 3.6 micrograms of D,L-thyroxine per 100 grams body weight per day as compared to control females which secreted 2.1 micrograms.

12. The thyroid secretion rate was not increased over control mature female mice at the twenty-first day of lactation although there is a possibility that the thyroid secretion rate may have been higher at an earlier stage in lactation.

13. Evidence is presented to show that thyroxine does not pass through the mammary gland of the mouse into the milk.

14. Lowering the environmental temperature below the zone of thermoneutrality in the mouse increased the thyroid secretion rate in both males and females. Thus, at 87° Fahrenheit mature Schwing male mice secreted the equivalent of 0.7 and females the equivalent of 3.2 micrograms of D,L-thyroxine per 100 grams body weight per day. At 80° Fahrenheit, male mice secreted the equivalent of 2.4 micrograms and the females secreted 5.5 micrograms of D,L-thyroxine per 100 grams body weight per day.

15. It was found that in mice approximately seven per cent of the thyroxine in thyroprotein is utilized when it is administered orally as compared to subcutaneous injection.

16. These studies emphasize the importance of using the normal thyroid secretion rate of animals as a reference base in studying the effects of hypo- or hyperthyroidism upon the various physiological processes of the animal body.

## ACKNOWLEDGMENT

The writers wish to express their appreciation to Professor A. C. Ragsdale, chairman of the Dairy Husbandry Department, for his interest and advice; to Mr. T. Y. Liu, Graduate Assistant, for his cooperation during the early phases of this work; to Mr. A. J. Olsan for his care of the laboratory animals; and to Jack Miller, Student Assistant.

Appreciation is also due the Cerophyl Laboratories, Kansas City, Missouri, for supplying the thyroprotein used in these studies; to the Lederle Laboratories of Pearl River, New York, for supplying thiouracil; and to Merck and Company, Rahway, New Jersey, for supplying dianisylhexene.

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